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MICRORNA-MEDIATED GENE REGULATION IN ASTHMA: MOLECULAR PATHOGENESIS, BIOMARKERS, AND THERAPEUTIC TRANSLATION

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ABSTRACT

Background: Asthma affects more than 300 million people worldwide and remains poorly controlled in a substantial proportion of patients despite available pharmacotherapy. Heterogeneity across allergic eosinophilic, neutrophilic, and mixed endotypes underscores the need for precise molecular tools. MicroRNAs (miRNAs)—small, ~22-nucleotide non-coding RNAs—have emerged as key modulators of immune polarization, airway epithelial integrity, airway smooth-muscle remodeling, and glucocorticoid responsiveness.

Objective: This narrative review examines the mechanistic roles of major asthma-associated miRNAs, evaluates circulating and extracellular-vesicle (EV) miRNAs as clinical biomarkers, and appraises preclinical therapeutic modulation strategies.

Key Findings: Among the most consistently implicated miRNAs, miR-21 promotes Th2 polarization via IL-12p35 suppression and amplifies steroid resistance through PI3K-mediated suppression of histone deacetylase 2 (HDAC2); it also enhances PI3K-Akt signaling by targeting PTEN, contributing to airway smooth-muscle proliferation and migration. miR-155 drives eosinophilic inflammation through PU.1 suppression, while miR-146a/b exert context-dependent anti-inflammatory effects that may be attenuated during viral exacerbations. The let-7 family maintains epithelial homeostasis and is downregulated in asthmatic airways. Biomarker studies in small case-control cohorts report potentially useful diagnostic signals (e.g., AUC = 0.91 for serum exosomal miR-155 in one moderate-to-severe asthma study); these are exploratory estimates from individual studies lacking external validation. Preclinical evidence supports antagomir-21 restoration of steroid sensitivity, miR-146a mimic attenuation of rhinovirus-induced inflammation, and mucus-penetrating EV delivery of miR-511-3p reversing allergic airway inflammation in murine models.

Conclusions: No completed asthma-specific miRNA therapeutic trial was identified. Translation requires multicenter biomarker validation, standardized pre-analytical workflows, human mechanistic studies, phenotype-stratified trial designs, and reproducible pulmonary delivery systems capable of cell-specific target engagement.

KEYWORDS: microRNA; asthma; non-coding RNA; post-transcriptional regulation; biomarkers; antagomir; extracellular vesicles; steroid resistance; nanoparticle delivery

1. INTRODUCTION

Asthma is a heterogeneous chronic inflammatory disease of the airways, characterized by variable and reversible airflow obstruction, airway hyperresponsiveness, hypersecretion of mucus, and progressive structural remodeling. Worldwide prevalence exceeds 300 million individuals, and the disease remains inadequately controlled in 5–10% of patients despite maximal pharmacotherapy [1]. Current therapeutic options—inhaled corticosteroids (ICS), long-acting beta-agonists (LABAs), and biologics targeting immunoglobulin E (anti-IgE), interleukin-5 (anti-IL-5), and the IL-4 receptor alpha subunit (anti-IL-4Rα)—reduce type 2-high inflammation but are largely ineffective in non-type 2 endotypes such as neutrophilic and paucigranulocytic asthma [2]. Steroid-resistant or corticosteroid-insensitive asthma accounts for a disproportionate share of asthma-related morbidity, hospitalizations, and healthcare costs, and its molecular underpinnings remain incompletely understood.

The clinical heterogeneity of asthma—spanning allergic eosinophilic, non-allergic eosinophilic, neutrophilic, and mixed phenotypes—reflects distinct and partially overlapping molecular pathways. This heterogeneity creates an unmet need for precision molecular diagnostics that can phenotype patients beyond peripheral blood eosinophil counts and fractional exhaled nitric oxide (FeNO), and for mechanistically informed therapeutic targets that extend beyond cytokine neutralization. MicroRNAs (miRNAs) are candidate solutions to both challenges.

MicroRNAs are endogenous, ~22-nucleotide non-coding RNA molecules that regulate gene expression post-transcriptionally by binding, with imperfect complementarity, to the 3′ untranslated regions (3′ UTRs) of target messenger RNAs (mRNAs), thereby inducing translational repression or mRNA degradation via the RNA-induced silencing complex (RISC) [3]. A single miRNA can regulate hundreds of transcripts, and a single transcript may be co-regulated by multiple miRNAs, creating complex and context-sensitive gene-regulatory networks [4]. In asthma, miRNAs have been implicated in T helper cell polarization, innate immune recognition of respiratory

viruses, epithelial barrier function, airway smooth-muscle proliferation, fibroblast activation, and glucocorticoid receptor signaling [5,6].

The stability of miRNAs in biological fluids—conferred by their encapsulation within extracellular vesicles (EVs), including exosomes, and by association with RNA-binding proteins such as Argonaute-2—has generated substantial interest in their use as non-invasive circulating biomarkers [7,8]. Unlike many protein biomarkers that are susceptible to enzymatic degradation and post-translational modification, miRNAs can be quantified reliably using standardized quantitative reverse-transcription PCR (qRT-PCR) and next-generation sequencing platforms [9].

The ability to pharmacologically modulate miRNA activity using synthetic oligonucleotides adds a direct therapeutic dimension. Antagomirs—chemically modified antisense oligonucleotides—silence overexpressed miRNAs, whereas miRNA mimics replace deficient ones, offering a means to correct pathological miRNA dysregulation [10]. Proof-of-concept preclinical studies have demonstrated that miRNA-targeted interventions can restore steroid sensitivity in neutrophilic asthma, attenuate allergen-induced eosinophilic inflammation, and augment antiviral responses in airway epithelial cells [11–13]. However, cell-type-specific delivery, off-target effects, immunogenicity, and pharmacokinetic barriers remain formidable challenges to clinical translation [14].

This review falls within the fields of non-coding RNA biology, post-transcriptional gene regulation, molecular pathogenesis, and translational molecular diagnostics. It examines how asthma-associated microRNAs regulate immune polarization, epithelial responses, glucocorticoid sensitivity, and airway remodeling, while assessing their development as circulating biomarkers and RNA-based therapeutic targets.

Specifically, this narrative review: (i) critically appraises mechanistic evidence for major asthma-associated miRNAs, distinguishing experimentally validated targets from predicted associations and correlational findings; (ii) evaluates the diagnostic accuracy and clinical utility of circulating and EV-derived miRNAs as candidate biomarkers; (iii) appraises preclinical evidence for antagomir, mimic, nanoparticle, EV, and inhalable therapeutic strategies; and (iv) identifies the translational, regulatory, and manufacturing barriers that must be addressed before miRNA-based diagnostics or therapeutics can enter clinical practice in asthma.

2. LITERATURE SEARCH STRATEGY

A structured narrative literature search was conducted using PubMed, Scopus, Web of Science, and Google Scholar. Search terms included combinations of "asthma," "microRNA," "miRNA," "non-coding RNA," "biomarker," "exosome," "extracellular vesicle," "antagomir," "miRNA mimic," "steroid resistance," "corticosteroid insensitivity," "airway remodeling," "RNA therapeutics," "nanoparticle," "lipid nanoparticle," "inhalable," "HDAC2," "PI3K," "NF-κB," "RISC," "Dicer," "Drosha," "let-7," "miR-21," "miR-155," "miR-146a," and "miR-511-3p." The search was not restricted by date but prioritized articles published between 2005 and 2026. No language restriction was applied, although only articles available in English were reviewed in detail.

Priority was given to original mechanistic studies, biomarker cohort studies, preclinical therapeutic studies, and pharmacological or delivery-focused studies, supplemented by high-quality reviews and consensus documents for contextual synthesis. Studies were considered if they addressed miRNA expression patterns, experimentally validated molecular targets, cell-signaling pathway associations, diagnostic performance metrics, therapeutic modulation strategies, or delivery systems relevant to asthma. Where clinical trial claims were made in the reviewed literature, trial registry databases (ClinicalTrials.gov) were consulted for context. This review is narrative in design and does not claim exhaustive or systematic coverage; study selection was not performed using formal PRISMA or PICOS criteria.

3. MicroRNA Biogenesis and Post-Transcriptional Gene Regulation

MicroRNA genes are typically transcribed by RNA polymerase II as long primary transcripts (pri-miRNAs) that form hairpin structures. Within the nucleus, the Microprocessor complex—comprising the RNase III enzyme Drosha and its cofactor DGCR8 (DiGeorge syndrome critical region gene 8)—cleaves pri-miRNAs to generate ~65-nucleotide precursor miRNAs (pre-miRNAs) [3]. Pre-miRNAs are exported to the cytoplasm by Exportin-5 in a RanGTP-dependent manner, where the RNase III enzyme Dicer cleaves them into ~22-nucleotide miRNA duplexes. One strand of the duplex—the guide strand—is preferentially loaded into the Argonaute (AGO) protein, the catalytic core of the RISC, while the complementary passenger strand is typically degraded [3].

Within the RISC, the guide miRNA strand directs AGO to target mRNAs through imperfect seed-region complementarity (nucleotides 2–8 from the 5′ end of the miRNA) with the 3′ UTR of the target transcript. The degree of complementarity determines the post-transcriptional outcome: near-perfect complementarity promotes endonucleolytic cleavage of the target mRNA, whereas partial complementarity induces translational repression and accelerated mRNA decay [3,4]. A single miRNA can repress hundreds of target transcripts with modest individual effects, enabling fine-tuning rather than binary switching of gene-expression programs. Conversely, multiple miRNAs may converge on a single transcript to achieve coordinated silencing of entire pathways [4].

Key regulatory checkpoints in miRNA biogenesis—including Drosha, Dicer, and DGCR8 expression—are subject to feedback regulation and can be disrupted by chronic inflammatory stimuli, hypoxia, and pharmacological agents relevant to asthma [5]. Altered expression of Dicer has been reported in asthmatic airways, suggesting that global

miRNA processing efficiency may be dysregulated in the disease, with downstream consequences for the entire miRNA regulatory network [6].

Non-canonical miRNA biogenesis pathways also exist. Mirtrons—short hairpin introns—bypass Drosha processing and are directly processed by Dicer, while some miRNAs arise from small nucleolar RNAs or other RNA species through Dicer-independent mechanisms [3]. These pathways may contribute to tissue-specific or condition-specific miRNA expression patterns relevant to asthma phenotype heterogeneity.

The post-transcriptional regulation mediated by miRNAs integrates with other gene-regulatory layers, including transcription factor networks, chromatin remodeling, and RNA-binding proteins. In asthma-relevant cells—dendritic cells, T helper cells, eosinophils, airway epithelial cells, airway smooth-muscle cells, and macrophages—miRNA-mediated networks create cell-type-specific regulatory hubs that integrate environmental signals (allergens, respiratory viruses, pollutants, glucocorticoids) into coordinated transcriptional and translational responses.

4. MicroRNA Regulatory Networks in Asthma Pathogenesis

Table 1 summarizes the major asthma-associated miRNAs, their validated molecular targets, principal signaling pathways, cell types, and biological roles. The following subsections discuss the five most mechanistically and clinically characterized miRNA families.

4.1. miR-21: Th2 Polarization, PI3K-Akt Signaling, HDAC2 Suppression, and Steroid Resistance

MicroRNA-21 (miR-21) is among the most consistently overexpressed miRNAs in asthmatic airways and is particularly elevated in severe, corticosteroid-insensitive subtypes across human cohorts and animal models [15,16]. Its pathogenic contribution to asthma spans immune polarization, structural airway remodeling, and pharmacological steroid resistance through distinct but converging molecular mechanisms.

In dendritic cells, miR-21 suppresses IL-12p35 expression, the limiting subunit of bioactive IL-12p70, thereby impairing Th1-skewing capacity and promoting relative Th2 preponderance [17]. This shift in T helper polarization favors the production of IL-4, IL-5, and IL-13, driving eosinophilic inflammation, IgE class switching, and hypersecretion of mucus. This mechanism was established in the allergic context by Lu et al. in a murine ovalbumin model [17].

In airway smooth-muscle cells, miR-21 can promote PI3K-Akt signaling by suppressing phosphatase and tensin homolog (PTEN), thereby contributing to airway smooth-muscle cell proliferation, migration, inflammatory signaling, and reduced glucocorticoid responsiveness [18]. PTEN is a lipid phosphatase that opposes the PI3K-Akt axis; its suppression by miR-21 constitutively activates downstream proliferative and survival signaling, a mechanism that is experimentally validated in human airway smooth-muscle cell lines [18]. This role is distinct from the immune-polarization function and positions miR-21 as a convergent driver of structural remodeling.

The most clinically consequential function of miR-21 in asthma may be its role in glucocorticoid resistance. Kim et al. demonstrated, in a murine model of steroid-insensitive neutrophilic asthma induced by co-infection with Streptococcus pneumoniae and Haemophilus influenzae, that elevated miR-21 amplifies PI3K-mediated suppression of histone deacetylase 2 (HDAC2) [11]. HDAC2 is essential for glucocorticoid receptor function; its inhibition by activated PI3K renders inflammatory gene-expression programs insensitive to corticosteroids. Importantly, systemic antagomir-21 administration restored HDAC2 expression, reduced airway hyperresponsiveness, and recovered steroid sensitivity in this model, enabling low-dose corticosteroids to suppress inflammation effectively [11]. A conference abstract from the same investigative group provided supporting immunological data [15]. These findings position miR-21 as a mechanistically plausible target for restoring steroid responsiveness in neutrophilic asthma.

As a circulating biomarker, serum miR-21 is elevated in asthmatic patients compared with healthy controls. Elbehidy et al. reported upregulation of serum miR-21 in asthmatic children with a correlation to disease severity [16]. ElKashef et al. reported, in a small case-control study involving 40 asthmatic adults and 20 controls, that serum miR-21 differentiated groups with an apparent sensitivity of 100% and specificity of 95% at a threshold of 1.37-fold change [19]; however, these estimates derive from a single small cohort without external validation and should be interpreted as preliminary exploratory data rather than established clinical performance metrics.

Clinically, the broad expression of miR-21 across multiple tissues and physiological processes raises concern for systemic off-target effects of miR-21 inhibition. This reinforces the need for lung-specific or airway-targeted delivery approaches, such as inhalable antagomir formulations, before therapeutic evaluation in humans.

4.2. miR-155: Eosinophilic Inflammation and Immune-Cell Regulation

MicroRNA-155 is consistently overexpressed in asthmatic airways and plays an essential mechanistic role in type 2-high, Th2-mediated eosinophilic inflammation. Malmhäll et al. demonstrated, using miR-155–deficient mice subjected to ovalbumin sensitization and challenge, that the absence of miR-155 markedly attenuated airway eosinophilia, Th2 cell accumulation, and Th2 cytokine production (IL-4, IL-5, IL-13) in bronchoalveolar lavage fluid [12]. The mechanistic basis involves miR-155–mediated suppression of PU.1, a transcription factor in myeloid and lymphoid lineages whose derepression limits the generation of eosinophil-attracting mediators including eotaxin and periostin.

In the biomarker domain, Khoie et al. isolated serum exosomes from 18 moderate-to-severe asthmatic patients and 18 healthy controls and reported that exosomal miR-155 and miR-221 were significantly elevated in the asthmatic

group [20]. The AUC for exosomal miR-155 was 0.91 (95% confidence interval not reported) and for miR-221 was 0.76, distinguishing asthmatics from controls; miR-221 also correlated positively with forced vital capacity [20]. These findings require interpretation within the context of the small sample size and the absence of an independent validation cohort. In a separate study, ElKashef et al. reported that serum miR-155 differentiated asthmatic patients from controls with an apparent sensitivity and specificity of 100% at a cut-off of 1.96, in a case-control study of 40 asthmatics and 20 healthy controls [19]; these figures should be regarded as exploratory estimates subject to optimism bias in small discovery cohorts.

Therapeutically, miR-155 inhibition has shown limited efficacy in pharmacological models. A review by Plank et al. noted that antagomir-155 administration in ovalbumin-sensitized mice reduced lung miR-155 levels but failed to produce a significant change in disease phenotype [21]. Cellular analysis attributed this discrepancy to selective uptake of the antagomir by myeloid cells (macrophages, neutrophils) with inadequate penetration into lymphocytes—the primary source of Th2 cytokines [21]. This cell-type delivery barrier is a recurring theme in miRNA therapeutics: reducing total tissue miRNA levels is insufficient unless delivery reaches the pathologically relevant cell type.

The contrast between the strong phenotype observed in genetic miR-155 knockout models and the negligible effect of pharmacological inhibition illustrates a critical gap: genetic deletion eliminates miR-155 function throughout development and across all cell types, whereas oligonucleotide pharmacology is constrained by delivery efficiency, cellular uptake kinetics, and the timing of administration relative to inflammatory priming. Bridging this gap requires targeted delivery technologies capable of reaching lymphoid cells in the lung.

4.3. miR-146a and miR-146b: NF-κB Regulation and Context-Dependent Effects

The miR-146 family—comprising miR-146a and miR-146b—occupies a complex and context-dependent position in asthma pathobiology, functioning as a negative-feedback regulator of NF-κB signaling in some contexts while potentially impairing antiviral immunity in others [22,23].

In the setting of allergic inflammation, miR-146a is induced in airway epithelial cells by inflammatory stimuli and acts as a negative regulator of the NF-κB pathway by targeting TRAF6 and IRAK1—two upstream transducers of Toll-like receptor (TLR) and IL-1 receptor signaling—thereby limiting the production of pro-inflammatory chemokines including CCL5, IL-8, and CXCL1 [24]. Lambert et al. demonstrated in human bronchial epithelial cells that miR-146a augments the anti-inflammatory effects of glucocorticoids, suggesting a role in steroid sensitivity [24]. Consistent with an anti-inflammatory function, Wang et al. showed that co-administration of a TLR agonist and synthetic miR-146a mimics attenuated ovalbumin-induced asthma in mice, reducing Th2 cytokines and airway inflammation [23].

The context-dependent complexity of miR-146a becomes apparent during viral exacerbations. Laanesoo et al. demonstrated that rhinovirus infection of human bronchial epithelial cells induces miR-146a expression, and that delivery of miR-146a mimics via cell-penetrating peptide (CPP) nanocomplexes intranasally suppressed pro-inflammatory chemokine production and increased interferon-λ expression [22]. However, genetic deficiency of miR-146a in mice was associated with exaggerated neutrophilic and Th1/Th17 responses in some viral challenge models, indicating that complete miR-146a ablation may impair antiviral immunity [22]. These findings suggest that therapeutic augmentation of miR-146a could reduce allergic airway inflammation but might simultaneously attenuate antiviral responses, raising the possibility of increased susceptibility to infection-triggered exacerbations. Patient stratification by asthma phenotype—allergic versus infection-prone—will be essential in any future clinical application.

As a biomarker, miR-146a is upregulated in plasma of asthmatic children and correlates with IL-13 levels and eosinophil counts, as reported by Hammad et al. in a pediatric cohort [25]. Wardzyńska et al. reported elevated serum miR-146a during asthma exacerbations in middle-aged and elderly patients, though the cohort was small (n = 17) and no AUC was reported [26]. The diagnostic utility of miR-146a as a standalone biomarker appears more modest than that of miR-21 or miR-155 based on available evidence.

4.4. let-7 Family: Epithelial Homeostasis and Th2-Associated Pathways

The let-7 family encompasses multiple members (including let-7a, let-7c, let-7f) that collectively function as regulators of epithelial homeostasis and inhibitors of Th2-associated signaling programs. Rijavec et al. reported by small RNA sequencing of bronchial biopsies from 79 asthmatic patients and controls that let-7a is downregulated in severe asthma and negatively correlates with airway eosinophilia and impaired lung function [27]. Roffel et al. similarly identified let-7 family downregulation in bronchial biopsies from asthmatic subjects, supporting a role in disease pathogenesis [28].

The mechanistic basis for let-7's protective role is incompletely characterized but involves regulation of IL-13 signaling pathways and control of pro-inflammatory gene expression in airway epithelial cells [29]. Reduced let-7 expression may allow upregulation of HMGA2 and other oncofetal targets that promote epithelial-to-mesenchymal transition, mucous cell metaplasia, and Th2-permissive gene programs. Circulating let-7a is downregulated in plasma of asthmatic children and has been evaluated as a candidate biomarker for diagnosis and severity prediction [30]. Dynamic changes in circulating exosomal let-7a and let-7f have also been documented during experimental rhinovirus challenge in asthmatic adults, implicating this family in antiviral exacerbation responses [31].

Therapeutically, miRNA mimic replacement of deficient let-7 is a rational but substantially underexplored strategy. No preclinical studies demonstrating efficacy of let-7 mimic administration in asthma models have been identified in the literature reviewed. The broad regulatory functions of let-7 family members across cell proliferation, differentiation, and metabolic homeostasis raise theoretical concerns regarding systemic off-target effects, underscoring the need for airway-targeted delivery strategies in any future let-7 replacement program.

4.5. miR-511-3p and Other Emerging Asthma-Associated miRNAs

MicroRNA-511-3p is encoded within the CD206 (MRC1) gene locus and is preferentially expressed in M2-polarized macrophages in the lung. Tu et al. demonstrated that miR-511-3p is downregulated in allergic asthma and that its restoration via mannose-decorated EVs loaded with miR-511-3p mimics and an RNA nanoparticle scaffold delivered intranasally reversed allergic airway inflammation in a murine model [13]. Complement component C3 was identified as a validated target of miR-511-3p, providing a mechanistic link between miR-511-3p loss, complement activation, and macrophage-mediated eosinophilic inflammation [13]. Rescue of the phenotype in miR-511-3p knockout mice confirmed on-target activity. This work also provided a proof-of-concept for mucus-penetrating EV delivery of miRNA to lung macrophages.

Additional emerging miRNAs of interest in asthma pathogenesis include miR-31-5p, which has been associated with airway smooth-muscle dysfunction; miR-126, implicated in vascular endothelial and eosinophil biology; and miR-221, identified in serum exosomes of moderate-to-severe asthmatic patients with correlation to lung function [20]. However, the mechanistic evidence for most emerging candidates remains primarily correlational or based on single studies, and their inclusion in diagnostic or therapeutic pipelines is premature without independent replication.

Figure 1 provides a schematic overview of the mechanistic roles of these key miRNAs in asthma pathogenesis, illustrating their convergent regulation of Th2 inflammation, NF-κB signaling, PI3K-Akt activation, epithelial integrity, and airway remodeling.

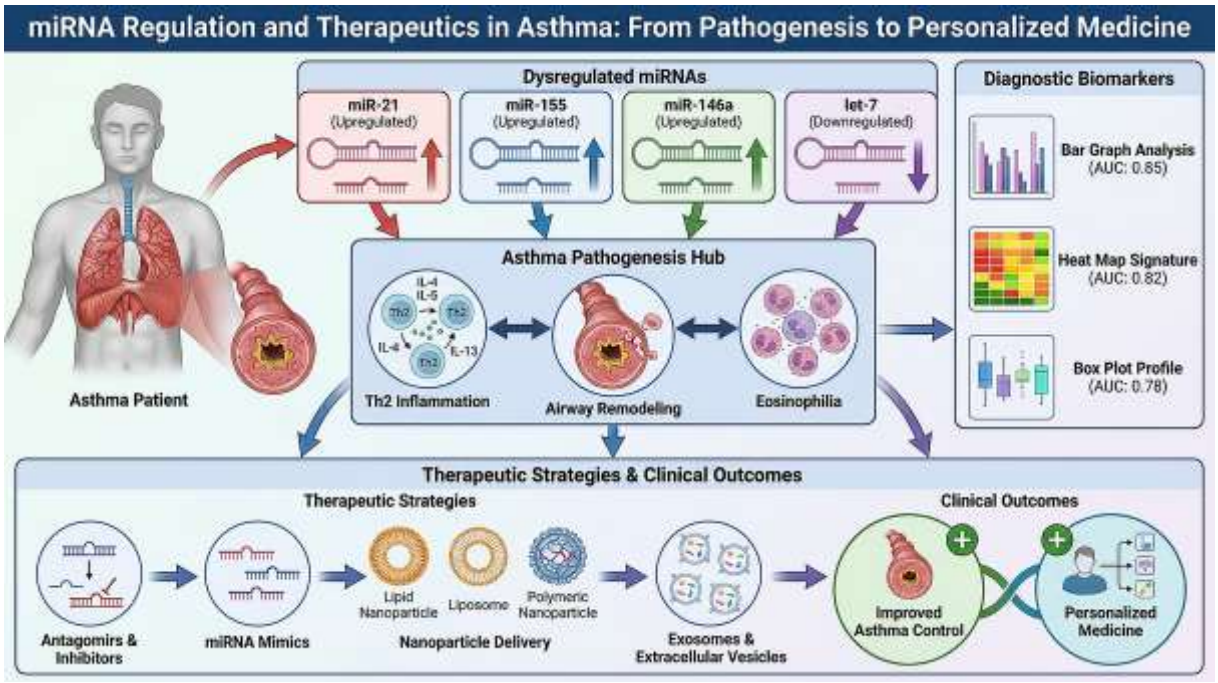

Figure 1. MicroRNA Regulatory Networks in Asthma Pathogenesis

Schematic overview of the major miRNA-mediated regulatory circuits operating in asthma-relevant cell types. In dendritic cells, miR-21 suppresses IL-12p35, impairing Th1 skewing and promoting relative Th2 polarization. In airway smooth-muscle cells (ASMCs), miR-21 suppresses PTEN, activating the PI3K-Akt axis and driving cell proliferation, migration, and reduced glucocorticoid responsiveness; PI3K-mediated phosphorylation additionally suppresses HDAC2, conferring steroid resistance. In Th2 cells and myeloid cells, miR-155 suppresses PU.1, facilitating the production of IL-4, IL-5, IL-13, eotaxin, and periostin, which collectively drive eosinophilic airway inflammation and IgE production. In airway epithelial cells, miR-146a negatively regulates the NF-κB pathway via suppression of TRAF6 and IRAK1, attenuating chemokine (CCL5, IL-8, CXCL1) production; this anti-inflammatory circuit may be attenuated during rhinovirus infection, potentially impairing antiviral interferon responses. The let-7 family maintains epithelial homeostasis by restraining Th2-permissive gene programs and opposing epithelial-to-mesenchymal transition; downregulation in asthmatic biopsies is associated with eosinophilia and impaired lung function. Downward-pointing arrows indicate suppression; upward-pointing arrows indicate activation or upregulation. Dotted arrows indicate proposed or correlational relationships. Solid

arrows indicate experimentally validated direct relationships in relevant cell types. Red labels: miRNAs upregulated in asthma. Blue labels: miRNAs downregulated in asthma.
Abbreviations: ASMC, airway smooth-muscle cell; CCL5, C–C motif chemokine ligand 5; CXCL1, C-X-C motif chemokine ligand 1; DC, dendritic cell; HDAC2, histone deacetylase 2; IgE, immunoglobulin E; IL, interleukin; IRAK1, IL-1 receptor-associated kinase 1; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; RISC, RNA-induced silencing complex; TRAF6, tumor necrosis factor receptor-associated factor 6; Th, T helper cell type

5. MicroRNAs Across Asthma Endotypes

5.1. Type 2-High and Eosinophilic Asthma

In type 2-high asthma—the dominant endotype characterized by elevated FeNO, eosinophilia, and Th2 cytokine excess—miR-21, miR-155, and miR-146a are collectively overexpressed, creating a regulatory circuit that amplifies IL-4/IL-5/IL-13 output and suppresses counter-regulatory Th1 signals. The convergence of these miRNAs on NF-κB, PI3K, and PU.1 pathways generates synergistic pro-inflammatory effects that may explain why type 2-high patients who do not respond to ICS or biologics have more complex molecular dysregulation than can be corrected by targeting a single cytokine. Serum and exosomal elevations of miR-21 and miR-155 are predominantly reported in eosinophilic phenotypes, supporting their phenotype specificity as biomarkers, although direct head-to-head comparisons with FeNO or periostin are lacking.

5.2. Neutrophilic and Steroid-Resistant Asthma

Neutrophilic asthma and steroid-resistant asthma represent the most clinically refractory subgroup, associated with Th17/Th1 skewing, neutrophil predominance, and PI3K-mediated HDAC2 suppression. miR-21 is particularly implicated in steroid resistance through the PTEN–PI3K–HDAC2 axis described in Section 4.1 [11]. Emerging evidence points toward additional miRNAs dysregulated in neutrophilic asthma, including members of the miR-17-92 cluster, though mechanistic validation in human neutrophilic asthma is limited. The therapeutic potential of antagomir-21 in steroid-resistant asthma is the strongest existing proof-of-concept for miRNA-targeted intervention with direct clinical relevance [11].

5.3. Virus-Associated Asthma Exacerbations

Respiratory virus-triggered exacerbations—predominantly rhinovirus-induced—account for the majority of severe asthma episodes and hospitalization. miR-146a, let-7f, let-7a, and miR-126-3p are dynamically regulated during rhinoviral challenge in asthmatic subjects [22,31]. Laanesoo et al. demonstrated that rhinovirus infection induces miR-146a in epithelial cells, which limits excessive chemokine production while augmenting interferon-λ responses—a context in which therapeutic augmentation of miR-146a may be beneficial [22]. However, as discussed in Section 4.3, context-dependent effects require careful patient stratification. Wang et al. reported longitudinally profiled exosomal miRNA dynamics during experimental rhinovirus challenge in both mild asthmatics and healthy controls, identifying miRNAs whose temporal expression patterns correlated with Th1/interferon or Th2/inflammatory signatures [31], suggesting potential utility as real-time exacerbation biomarkers, though diagnostic cutoffs have not been established.

5.4. Airway Remodeling and Smooth-Muscle Dysfunction

Airway remodeling—encompassing subepithelial fibrosis, goblet-cell hyperplasia, smooth-muscle hypertrophy, and increased vascular density—is poorly addressed by current anti-inflammatory therapies. miR-21 promotes airway smooth-muscle cell (ASMC) proliferation and migration by suppressing PTEN and activating PI3K-Akt in human ASMC lines [18]. Let-7 family members oppose epithelial-to-mesenchymal transition, and their downregulation in asthmatic biopsies may facilitate pathological epithelial plasticity [27,28]. miR-155 contributes indirectly to remodeling through persistent eosinophilic tissue injury. The remodeling context is particularly relevant for therapeutic targeting, as interventions that normalize smooth-muscle biology—rather than simply suppressing acute inflammation—could provide disease-modifying effects not achievable by cytokine blockade.

6. Circulating and Extracellular-Vesicle MicroRNAs as Biomarkers

6.1. Serum and Plasma miRNAs

Serum and plasma miRNAs have been investigated as non-invasive biomarkers of asthma diagnosis, phenotyping, and treatment-response monitoring across multiple studies. The biological rationale rests on the demonstrated stability of miRNAs in biological fluids—owing to EV encapsulation and protein-binding—and the sensitivity of detection platforms based on qRT-PCR, digital PCR, or next-generation sequencing [9].

The most studied candidates in serum and plasma are miR-21 and miR-155. ElKashef et al. evaluated a small case-control cohort of 40 asthmatic adults and 20 healthy controls and reported that serum miR-21 differentiated groups with an apparent sensitivity of 100% at a threshold of 1.37 and miR-155 at a cut-off of 1.96 with apparent sensitivity and specificity both reaching 100% [19]. These estimates should be interpreted with caution: the case-control design—comparing clearly asthmatic patients against healthy volunteers—inflates diagnostic performance relative to the clinically relevant scenario of differentiating asthma from other respiratory conditions (e.g., COPD, chronic bronchitis). Small sample sizes and the absence of external validation in independent cohorts compound these concerns. These figures therefore represent preliminary exploratory data, not validated clinical performance metrics.

In a pediatric cohort, Hammad et al. reported significant upregulation of plasma miR-21 and miR-146a in asthmatic children compared with healthy controls, with correlations to IL-13 and eosinophil counts, though no AUC or other formal diagnostic performance metric was presented [25]. Karam et al. identified miR-155 and let-7a in plasma as candidate biomarkers for childhood asthma and severity prediction, with ROC curve analysis performed, but reproducible performance measures were not detailed in the available literature [30].

The convergence of directionally consistent results—miR-21 and miR-155 upregulation, let-7 downregulation—across multiple independent studies in different populations lends biological plausibility to these candidates as genuinely dysregulated miRNAs in asthma, and distinguishes them from assay-specific technical artifacts. However, this convergence is necessary but not sufficient to establish clinical biomarker utility.

6.2. Exosomal and Salivary miRNAs

Extracellular vesicles—particularly exosomes (30–150 nm diameter, released through the multivesicular endosomal pathway)—offer miRNA cargo that is both highly stable and potentially reflective of the cellular origin and functional state of EV-producing cells in lung tissue [34,35]. The term 'exosome' is used here for vesicles that meet operational characterization criteria consistent with MISEV2018 guidance (ultracentrifugation-based isolation, electron microscopy/nanoparticle tracking analysis sizing, and tetraspanin marker verification); studies that did not perform this level of characterization are described as reporting EV-associated miRNAs.

Khoie et al. isolated serum exosomes from 18 moderate-to-severe asthmatic patients and 18 healthy controls and reported AUCs of 0.91 for exosomal miR-155 and 0.76 for miR-221 in distinguishing groups [20]. These preliminary results indicate discriminatory potential but require replication in larger, prospectively enrolled cohorts with clinical phenotyping.

Khalyfa et al. characterized exosomal miRNA profiles in plasma and saliva from pediatric asthmatic patients stratified by lung function (severe airflow obstruction versus normal) [36]. Twenty-seven differentially expressed miRNAs were identified in plasma exosomes and 40 in salivary exosomes, with pathway enrichment implicating T-cell and B-cell signaling networks [36]. The non-invasive saliva approach is particularly relevant for pediatric populations in whom repeated venipuncture is undesirable. No AUC values were reported for individual miRNAs, limiting formal diagnostic performance assessment.

Wang et al. conducted a longitudinal profiling of circulating exosomal miRNAs during experimental rhinovirus challenge in mild asthmatic adults and healthy controls [31]. Twenty-six exosomal miRNAs—including let-7a, let-7f, miR-122-5p, miR-126-3p, and miR-101-3p—were differentially expressed and displayed dynamic temporal patterns correlating with Th1/interferon or Th2/inflammatory transcriptional signatures [31]. These data suggest that EV miRNA dynamics may track immune responses during exacerbation, with potential as real-time monitoring tools.

6.3. Diagnostic Accuracy and Clinical Utility

Table 2 summarizes the diagnostic performance of major miRNA biomarker studies. Across the available literature, the most consistently observed limitation is the small sample size (typically 17–40 participants per group), which limits statistical power and increases the risk of effect-size inflation. The 'winner's curse' phenomenon—wherein true association estimates are systematically overestimated in initial discovery studies—is well-documented in biomarker research [37] and is particularly relevant to the field of circulating miRNAs, where most published studies are underpowered discovery cohorts.

Additional critical limitations include: (i) the lack of external validation in independent prospectively enrolled cohorts; (ii) case-control designs that inflate apparent diagnostic accuracy by excluding disease-mimicking comparators (COPD, bronchitis, upper airway disease); (iii) the absence of head-to-head comparisons with established clinical biomarkers (FeNO, serum periostin, blood eosinophil counts); (iv) variability in biomarker definitions (total serum, plasma, exosomal, or EV-associated fraction); and (v) heterogeneity in asthma phenotypes and severity across study cohorts.

For miRNA biomarkers to achieve clinical utility, multicenter prospective cohort studies—enrolling diverse populations by age, ethnicity, and asthma endotype, applying standardized pre-analytical and analytical protocols, and including clinically relevant comparators—are required. Biomarker qualification should follow established frameworks, reporting confidence intervals for sensitivity, specificity, AUC, and predictive values, and should explicitly distinguish stable disease from exacerbation states.

6.4. Pre-Analytical and Analytical Standardization

Pre-analytical variables exert substantial influence on circulating miRNA measurements and represent a major source of inter-study inconsistency. Hemolysis—whether occurring in vivo or during sample processing—releases erythrocyte-derived miRNAs (particularly miR-451a and miR-23a-3p) into serum or plasma, confounding measurements [54]. Platelet contamination introduces platelet-derived miRNAs that are not reflective of the disease compartment of interest. Anticoagulant choice (EDTA versus citrate versus heparin) affects RNA yield and PCR inhibition; heparin in particular is known to inhibit reverse transcriptase and PCR amplification [33].

Sample handling protocols—including time from blood draw to serum/plasma separation, the number of freeze-thaw cycles, storage temperature (–20°C versus –80°C), and RNA extraction kit platform—each contribute independently to measurement variability and require standardization through reference standard operating procedures [33]. RNA extraction methods differ substantially in their efficiency and selectivity for small RNAs, and comparisons between studies using different extraction platforms are inherently confounded [55,58].

Normalization strategies for circulating miRNA quantification remain a subject of active debate. Endogenous reference miRNAs (e.g., miR-16, miR-93) cannot be assumed to be stable across health-disease comparisons [55]. Exogenous spike-in controls (e.g., cel-miR-39) control for extraction efficiency but not for biological pre-analytical variation. Total RNA normalization may be affected by the variable cellular content of serum or plasma. The miRNA Quality Control (miRQC) study systematically compared multiple quantification platforms and identified significant inter-platform variation even for the same samples [57], highlighting the importance of platform consistency within and across studies.

Where extracellular vesicles are studied, reporting should conform to MISEV2018 guidelines [34], including vesicle quantification, size distribution characterization, tetraspanin marker verification, and explicit statement of the isolation method used. Studies that do not perform this characterization should describe their preparations as 'EV-enriched' or use terminology appropriate to the isolation procedure.

7. Therapeutic Modulation of MicroRNAs

7.1. Antagomirs and Antisense Inhibitors

Antagomirs are single-stranded antisense oligonucleotides designed to silence overexpressed miRNAs through stoichiometric binding and steric blocking of the guide strand or by facilitating RISC disassembly and target miRNA degradation [38]. Chemical modifications—including 2′-O-methyl, 2′-O-methoxyethyl (2′-MOE), locked nucleic acid (LNA), and phosphorothioate backbone substitutions—improve nuclease resistance, binding affinity for the target miRNA, and reduce immunostimulatory potential [39].

The strongest preclinical evidence for antagomir efficacy in asthma involves miR-21. Kim et al. demonstrated that systemic antagomir-21 administration in a murine model of steroid-insensitive neutrophilic asthma restored HDAC2 expression, reduced PI3K pathway activity, decreased airway hyperresponsiveness, and recovered steroid sensitivity—allowing low-dose corticosteroids to effectively suppress inflammation [11]. This proof-of-concept suggests that antagomir-21 could serve as a steroid-sensitizing adjunct in refractory neutrophilic asthma, though the leap from murine model to human therapy requires extensive pharmacological and toxicological characterization.

By contrast, pharmacological inhibition of miR-155 with antagomirs has yielded more limited results. As reviewed by Plank et al., antagomir-155 reduced whole-lung miR-155 levels in ovalbumin-sensitized mice but did not significantly alter disease phenotype, attributed to selective myeloid-cell uptake with insufficient lymphocyte penetration [21]. This fundamental cell-type delivery problem—a consequence of the intrinsic pharmacological properties of the oligonucleotide formulation rather than the target miRNA biology—illustrates that tissue-level miRNA knockdown is not sufficient to predict therapeutic efficacy.

Challenges specific to antagomir-based therapy include: (i) off-target effects from the ubiquitous expression of therapeutically relevant miRNAs (e.g., miR-21) across non-pulmonary tissues when administered systemically; (ii) inadequately characterized pharmacokinetics in humans, including tissue distribution, intracellular half-life, and the duration of miRNA silencing after a single dose; (iii) the potential for innate immune activation by oligonucleotide sequences despite chemical modification; and (iv) the high cost of clinical-grade oligonucleotide synthesis, which may limit accessibility.

7.2. miRNA Mimics and Replacement Strategies

For miRNAs that are downregulated in asthma—particularly let-7 family members and miR-511-3p—miRNA mimic replacement therapy offers a logical therapeutic strategy. miRNA mimics are synthetic double-stranded RNA oligonucleotides that recapitulate the guide strand of the endogenous miRNA and, upon cellular delivery, are loaded into RISC to silence target transcripts [40].

Proof-of-concept for miR-146a mimic therapy has been provided by Wang et al., who showed that co-administration of a TLR agonist with synthetic miR-146a mimics attenuated ovalbumin-induced asthma in mice, reducing Th2 cytokines and airway inflammation [23]. The TLR agonist component was proposed to facilitate cellular uptake and enhance immunomodulatory context, suggesting that combination formulations may be superior to mimic delivery alone. In the context of virus-associated asthma, Laanesoo et al. demonstrated that intranasal delivery of miR-146a mimics via CPP nanocomplexes reduced pro-inflammatory chemokines and increased interferon-λ expression in rhinovirus-infected human bronchial epithelial cells [22].

No preclinical studies establishing efficacy of let-7 mimic replacement in asthma-specific models have been identified in the literature reviewed. This gap likely reflects concerns about the pleiotropic regulatory roles of let-7 family members in cell proliferation, differentiation, and metabolic homeostasis, which could translate into significant off-target effects with systemic delivery. Tissue-targeted or airway-restricted delivery approaches will be necessary to assess the therapeutic-to-toxicity ratio of let-7 replacement.

7.3. Nanoparticle Delivery Systems

Nanoparticle-based delivery platforms provide protection of oligonucleotides from nuclease degradation, enhance cellular uptake, facilitate endosomal escape, and offer opportunities for surface modification to achieve cell-type-specific targeting [41,42]. Several classes of nanoparticles have been explored or are directly relevant to miRNA delivery in asthma.

Lipid nanoparticles (LNPs), widely validated through mRNA vaccine applications including COVID-19 vaccines, are capable of efficiently encapsulating oligonucleotides and mediating cytoplasmic delivery through ionizable

lipid-mediated endosomal escape [43]. While no asthma-specific LNP-miRNA formulation has been tested in clinical trials, the platform's established safety and efficacy profile for systemic and intramuscular RNA delivery provides a foundation for adaptation to pulmonary delivery. LNP formulation optimization for inhaled delivery to airway cells remains an active area of investigation.

CPP nanocomplexes have been used by Laanesoo et al. for intranasal miR-146a mimic delivery to human bronchial epithelial cells and in a murine house dust mite model, demonstrating proof-of-concept for epithelial-targeted delivery [22]. The scalability and immunogenicity of CPP formulations require further characterization before clinical development.

Polymeric nanoparticles—including chitosan-based and poly(lactic-co-glycolic acid) (PLGA)-based systems—have been investigated for miRNA and oligonucleotide delivery across a range of biomedical applications [42]. These platforms offer controlled release kinetics and relative biocompatibility. Challenges include heterogeneous particle size distribution, manufacturing scale-up, and variable oligonucleotide encapsulation efficiency.

Surface-functionalized nanoparticles bearing cell-targeting ligands offer the prospect of cell-type-selective delivery. Tu et al. exploited mannose receptor expression on M2 lung macrophages to target mannose-decorated EVs to these cells for miR-511-3p delivery [13]. Nanoparticles functionalized with anti-CD4, anti-SIGLEC-8, or other lymphocyte-directed ligands could in principle redirect antagomir delivery to T cells or eosinophils, addressing the cellular delivery barrier that limited antagomir-155 efficacy.

7.4. Extracellular-Vesicle Delivery

Extracellular vesicles—particularly engineered exosomes—have emerged as natural nanocarriers for miRNA delivery, offering intrinsic biocompatibility, low immunogenicity, the capacity to traverse biological barriers, and inherent cell-type tropism conferred by their surface protein composition [45,46]. These properties make EVs attractive alternatives to synthetic nanoparticles, particularly for airway delivery applications where immune activation is a concern.

Tu et al. demonstrated the therapeutic utility of EV-based miRNA delivery in asthma using mannose-decorated EVs loaded with miR-511-3p mimics and an RNA nanoparticle scaffold [13]. Intranasally administered, these engineered EVs penetrated airway mucus, delivered functional miR-511-3p to lung macrophages, validated complement C3 as the operative molecular target, and reversed allergic airway inflammation in a murine model. Phenotypic rescue in miR-511-3p knockout mice confirmed on-target activity [13].

[NOTE FOR AUTHOR REVIEW — Reference verification required: The original manuscript cited a cross-linked multilamellar liposome cancer-chemotherapy study (Liu et al., Mol Pharm 2014) in support of the claim that hucMSC-derived EVs expressing miR-146a-5p attenuated epithelial inflammation. This citation was identified as misattributed and has been removed from the present revision. The claim regarding hucMSC-EVs with miR-146a-5p requires verification against the correct primary source before reinclusion.]

EV-based delivery faces formidable manufacturing challenges: current production methods (cell culture supernatant harvest, sequential ultracentrifugation) are labor-intensive, poorly scalable, and yield heterogeneous preparations with batch-to-batch variability [47]. Efficient loading of exogenous oligonucleotides into EVs remains technically demanding; electroporation-based loading, widely used, has been shown to cause oligonucleotide precipitation rather than genuine intraluminal loading in some settings [48]. Regulatory classification of engineered EVs—as advanced therapy medicinal products (ATMPs) in the European Union or as biological products by the FDA—creates high compliance burdens for manufacturing, characterization, and clinical-grade production.

7.5. Inhalable Pulmonary Formulations

Inhaled delivery of miRNA therapeutics to the lung offers a mechanistically logical route for asthma treatment, providing direct access to the disease compartment while minimizing systemic exposure [49]. This approach is consistent with the established paradigm of inhaled pharmacotherapy in asthma (ICS, LABAs, short-acting bronchodilators) and has potential for patient acceptability.

The development of inhalable miRNA formulations faces several distinct technical barriers. First, oligonucleotides must withstand the aerosol generation process (shear stress, temperature gradients, dehydration), which can degrade sensitive RNA-based molecules; dry powder formulations produced by lyophilization or spray-drying may stabilize oligonucleotides but risk structural disruption during atomization [50,52]. Second, adequate pulmonary deposition requires aerodynamic particle sizes of 1–5 μm for peripheral airway targeting or slightly larger for central airway delivery; formulation of lipid- or polymer-encapsulated oligonucleotides within this aerodynamic envelope is technically demanding [49,52]. Third, mucus penetration—particularly relevant in asthma, in which goblet-cell hyperplasia and mucociliary dysfunction increase mucus viscosity—requires either mucus-penetrating particle designs (dense pegylated particles) or mucus-dissolving co-formulation strategies [51]. Fourth, cellular uptake of naked oligonucleotides in airway epithelium is inherently low; nanoparticle or liposomal encapsulation substantially enhances transfection efficiency but introduces formulation complexity [53].

Liposomal formulations for inhaled drug delivery have been clinically validated for small-molecule drugs (e.g., liposomal amikacin), providing regulatory precedent and formulation knowledge that may be adaptable to oligonucleotide payloads [53]. Inhalable delivery of miRNA therapeutics thus represents the most clinically relevant long-term route for asthma treatment, but successful development will require close collaboration among oligonucleotide chemists, formulation scientists, aerosol engineers, and pulmonary clinicians (figure 2).

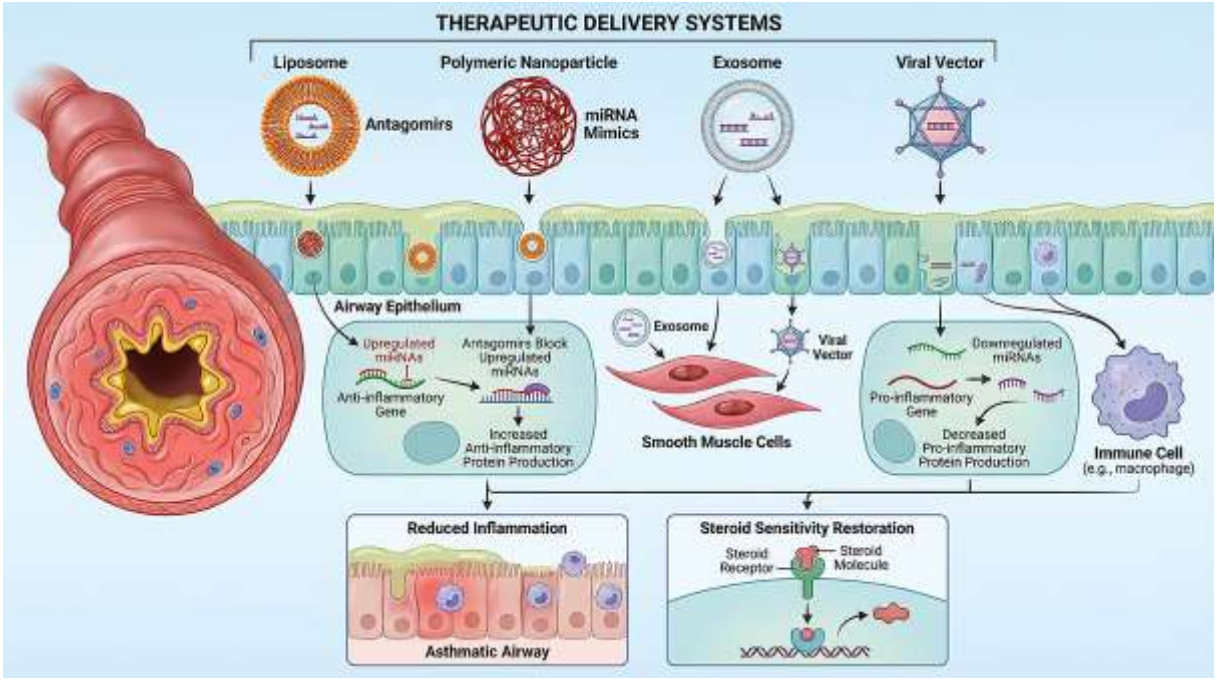

Figure 2. Therapeutic Modulation Strategies and Delivery Systems for miRNA Therapeutics in Asthma

Schematic representation of the principal approaches to therapeutic miRNA modulation and their delivery platforms in the context of asthma. Left panel: Therapeutic strategies. Antagomirs (antisense oligonucleotides) silence overexpressed miRNAs (e.g., miR-21, miR-155) by stoichiometric binding and blocking of RISC-mediated target repression. miRNA mimics restore deficient miRNAs (e.g., let-7, miR-511-3p, miR-146a) by delivering synthetic double-stranded RNA that is loaded into RISC and directs target mRNA silencing. Right panel: Delivery platforms. Lipid nanoparticles (LNPs) encapsulate oligonucleotides and mediate cytoplasmic delivery via ionizable lipid-mediated endosomal escape. Polymeric nanoparticles (e.g., PLGA, chitosan) provide controlled release and protection from nuclease degradation. Extracellular vesicles (EVs)—including engineered exosomes and mesenchymal stem cell-derived EVs—carry miRNA cargo with intrinsic biocompatibility and cell-tropism; surface functionalization with mannose or other targeting ligands enables cell-type specificity. Inhalable formulations (dry powder, nebulized LNPs, liposomal aerosols) deliver oligonucleotides directly to the airway, maximizing local concentration and minimizing systemic exposure, but must penetrate mucus (1–5 μm aerodynamic particle size), avoid mucociliary clearance, and achieve intracellular uptake in epithelial and immune cells. Translational limitations listed for each platform include endosomal escape efficiency, manufacturing scale-up, batch-to-batch consistency, target-cell specificity, off-target gene regulation, pharmacokinetics, immunogenicity, and the absence of clinical trial validation in asthma.
Abbreviations: ATMP, advanced therapy medicinal product; EV, extracellular vesicle; LNP, lipid nanoparticle; PLGA, poly(lactic-co-glycolic acid); RISC, RNA-induced silencing complex.
Note: Figures 1 and 2 are conceptual scientific illustrations. They should be prepared as editable vector graphics at a minimum resolution of 300 dpi for submission. All labeled interactions should distinguish experimentally validated relationships from proposed or correlational associations using distinct visual notation (solid versus dashed lines) consistent with the legends above.

8. Translational, Regulatory, and Manufacturing Challenges

8.1. Cell-Type-Specific Delivery

The most immediate translational barrier for miRNA therapeutics in asthma is the inability of current delivery systems to achieve sufficient intracellular concentrations in the target cell populations. Asthma is a multicellular disease in which different cell types predominate across phenotypes and disease phases: Th2 cells and eosinophils in type 2-high allergic asthma; neutrophils and Th17 cells in neutrophilic asthma; airway epithelial cells during viral exacerbations; and airway smooth-muscle cells and fibroblasts in remodeling. A delivery system optimized for one cell type is unlikely to be effective across all contexts, and the dynamic shift in cellular dominance between stable disease and exacerbation further complicates cell-targeting strategies. Combination delivery systems targeting multiple cell populations simultaneously increase complexity and cost.

8.2. Off-Target Effects and Immunogenicity

Oligonucleotide therapeutics retain intrinsic risks of off-target gene regulation, particularly for miRNAs with hundreds of predicted or validated transcriptome-wide targets. Partial seed-region complementarity means that a

therapeutic antagomir or mimic may inadvertently de-repress or repress unintended transcripts, with unpredictable biological consequences. Additionally, single-stranded and double-stranded oligonucleotides can activate Toll-like receptor 7/8 (TLR7/8) or cytosolic innate immune sensors (RIG-I, MDA5), triggering interferon responses and systemic inflammation. Chemical modifications reduce but do not eliminate immunostimulatory potential, and species differences in TLR sensitivity between mice and humans limit the extrapolability of murine immunogenicity data [61].

8.3. Pharmacokinetics and Biodistribution

The pharmacokinetic behavior of oligonucleotides—including plasma half-life, tissue distribution, intracellular residence time, and the duration of miRNA silencing after a single dose—is incompletely characterized for most miRNA-targeted therapeutic candidates and has not been established in human subjects in the asthma context. Approved oligonucleotide drugs such as mipomersen, nusinersen, and patisiran exhibit complex multiphasic pharmacokinetics governed by tissue binding, renal clearance, and endosomal recycling [60,62,63,65]. The lung presents additional pharmacokinetic complexity due to mucociliary clearance, macrophage-mediated phagocytosis of inhaled particles, and the epithelial barrier to submucosal delivery.

8.4. Manufacturing, Quality Control, and Scale-Up

The synthesis of therapeutic-grade oligonucleotides at clinical and commercial scale is costly and technically demanding, requiring multi-step phosphoramidite synthesis, purification by high-performance liquid chromatography or ion-exchange chromatography, and characterization by mass spectrometry for sequence confirmation and impurity profiling [63]. Engineered EV products face additional manufacturing complexity, including cell banking, production bioreactor design, isolation protocol standardization, size-exclusion and density-gradient characterization, and functional potency assays. Achieving batch-to-batch consistency for EV therapeutics—a prerequisite for regulatory approval—remains a central manufacturing challenge. The intellectual property landscape for oligonucleotide therapeutics is congested with patents covering chemical modifications, delivery vehicles, and specific sequences, adding legal complexity to commercial development [64].

8.5. Clinical-Trial and Regulatory Considerations

As of the most recent registry and literature search performed for this review, no completed asthma-specific clinical trial of a miRNA-targeted therapeutic was identified. This translational gap reflects the early state of the field rather than established safety or efficacy concerns, as first-in-human trials of several miRNA therapeutics in other disease contexts have been conducted, including RG-101 (anti-miR-122 for hepatitis C) and cobomarsen (anti-miR-155 for T-cell lymphoma).

Regulatory development of miRNA therapeutics will require Phase 1 trials establishing safety, pharmacokinetics, and pharmacodynamics; Phase 2 trials assessing efficacy against clinically meaningful endpoints (exacerbation rate, lung function, symptom scores, steroid-sparing); and biomarker-informed patient stratification strategies. The FDA and EMA have issued guidance for oligonucleotide drugs, but specific frameworks for miRNA therapeutics in inhaled pulmonary applications are still evolving [59]. Adaptive trial designs and biomarker-defined enrollment criteria—identifying patients with demonstrable target miRNA dysregulation—could accelerate development by enriching for responsive populations. The approved precedents of patisiran (siRNA for hereditary transthyretin amyloidosis) [65] and nusinersen (antisense oligonucleotide for spinal muscular atrophy) demonstrate that oligonucleotide drugs can achieve clinical approval, providing a regulatory blueprint for the field.

9. Future Research Priorities

Translating the substantial body of preclinical miRNA research into clinical benefit for asthma patients will require coordinated progress across several research domains.

1. Multicenter biomarker validation: Future biomarker studies must be designed from the outset as multicenter, prospective cohort investigations enrolling diverse patient populations by age, ethnicity, disease severity, and asthma endotype. Standardized protocols for sample collection, processing, and storage—ideally developed by consensus working groups—should be applied uniformly. External validation cohorts, enrolled independently of the discovery cohort, and pre-registration of the primary biomarker hypothesis are necessary to generate trust in reported diagnostic performance estimates. Head-to-head comparison with established biomarkers (FeNO, blood eosinophils, periostin) should be a standard design element.

2. Human mechanistic studies: The bulk of current mechanistic evidence derives from murine models or in-vitro cell systems, which differ from human asthma in immune architecture, airway anatomy, viral susceptibility, and glucocorticoid pharmacology. Human bronchial biopsies, bronchoalveolar lavage samples, and induced sputum should be systematically analyzed for miRNA expression by cell type, with integration of single-cell RNA sequencing to resolve cell-type-specific regulatory networks. Human organoid and air-liquid interface models may provide intermediate platforms between cell lines and in-vivo studies.

3. Advanced delivery system development: Targeted nanoparticles, engineered EVs, and inhalable formulations must be developed to achieve adequate and reproducible delivery to the relevant cell populations in the diseased airway. Specific design objectives include: aerodynamic characteristics for peripheral airway deposition; mucus-penetrating surface chemistry; cell-type-targeting ligands; efficient endosomal escape; and favorable immunogenicity and toxicology profiles. Scalable manufacturing processes compatible with current good manufacturing practice (cGMP) are an essential parallel objective.

4. Patient stratification strategies: Given the context-dependent functions of several key miRNAs (notably miR-146a), clinical development programs will need patient stratification by asthma endotype and molecular phenotype—allergic versus neutrophilic, infection-prone versus stable, steroid-resistant versus steroid-sensitive. Single-cell and multi-omic profiling of asthmatic airways in human biopsy material is needed to define the miRNA regulatory landscape specific to each endotype.
5. Combination therapies: miRNA-targeted agents may achieve greater efficacy as adjuncts to existing interventions. Antagomir-21 to restore steroid sensitivity combined with low-dose ICS, or miR-146a mimic combined with inhaled corticosteroids in allergic asthma, represents a rationally designed combination strategy with mechanistic synergy. Trials investigating such combinations will need appropriate factorial designs.
6. First-in-human clinical trials: Phase 1 safety and pharmacokinetic studies of miRNA-targeted therapeutics are urgently needed in asthma to characterize safety, pharmacodynamics (e.g., target miRNA level changes, downstream gene-expression biomarkers), and lung tolerability of inhaled formulations. Transparent collaboration with regulatory agencies from the early development stage will facilitate efficient trial design and define acceptable endpoints for Phase 2 efficacy evaluation.
7. Health economic evidence: Cost-effectiveness assessments comparing miRNA therapeutics to existing biologics will be necessary for payer acceptance. Given the high cost of oligonucleotide synthesis, demonstration of superiority in patient subgroups unmet by current therapies (steroid-resistant, neutrophilic asthma) will be essential to justify the investment.

10. CONCLUSIONS

MicroRNAs are biologically credible regulators of asthma pathogenesis and candidate biomarkers of disease phenotype, severity, and treatment response. The mechanistic evidence is most robust for miR-21, which amplifies steroid resistance through the PTEN–PI3K–HDAC2 axis and promotes Th2 polarization via IL-12p35 suppression; for miR-155, which drives eosinophilic inflammation through PU.1 targeting; and for the let-7 family, whose downregulation is associated with compromised epithelial homeostasis. miR-146a occupies a context-dependent position as an NF-κB brake in allergic inflammation and a potentially adverse regulator during viral exacerbations.
Current biomarker evidence remains limited by small cohorts, heterogeneous sampling and normalization procedures, and the near-absence of external validation. Reported diagnostic performance metrics—including individual studies reporting sensitivities and specificities near 100%—must be interpreted as exploratory estimates from discovery cohorts rather than established clinical performance, and do not yet support implementation of miRNA-based diagnostics in clinical practice.
Therapeutic modulation using antagomirs, mimics, nanoparticles, and extracellular vesicles has shown proof-of-concept efficacy primarily in preclinical models, but cell-specific delivery, pharmacokinetics, off-target effects, manufacturing, and regulatory requirements remain unresolved. No completed asthma-specific clinical trial of a miRNA-targeted therapeutic was identified in the literature reviewed.
Progress toward clinical translation will require standardized analytical workflows, multicenter validation, human mechanistic studies, phenotype-stratified trial designs, and pulmonary delivery systems capable of reproducible target engagement.

Declarations

Ethics Approval: Not applicable. This article is a narrative review of previously published literature.
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— [Reference 20 in original manuscript was a duplicate of reference 12. Removed and renumbered. All citations formerly attributed to [20] (Malmhäll et al. 2014) now cite [12] in this revised version.] —
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— [Original reference 42 (Liu Y et al., Mol Pharm 2014, 'Codelivery of doxorubicin and paclitaxel by cross-linked multilamellar liposome') was identified as a misattributed cancer-chemotherapy citation, incorrectly supporting claims about hucMSC-EV-mediated miR-146a-5p delivery in asthma. This reference has been removed. The author should identify and substitute the correct primary source for the hucMSC-EV miR-146a-5p study.] —
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Table 1. Major Asthma-Associated miRNAs: Validated Molecular Targets, Pathways, Cell Types, and Biological Roles

miRNA	Expression in asthma	Biological sample / model	Validated target(s)	Principal pathway(s)	Functional role	Evidence type	Key references
miR-21	Upregulated	Asthmatic airways (human biopsy, bronchoalveolar lavage); murine OVA and neutrophilic	IL-12p35 (dendritic cells); PTEN (airway smooth-muscle cells); HDAC2	PI3K–Akt–HDAC2; Th1/Th2 polarization	Suppresses Th1 skewing via IL-12p35 repression; promotes ASM proliferation and migration via PTEN	Murine KO and pharmacological antagomir model; human ASMC in vitro; human	[11, 16, 17, 18]

		models; serum	(indirect, via PI3K)		suppression/PI3K-Akt activation; amplifies steroid resistance through PI3K-mediated HDAC2 inhibition	biomarker cohort studies	
miR-155	Upregulated	Asthmatic airways (murine); serum/serum exosomes (human case-control)	PU.1 (transcription factor in myeloid and lymphoid lineages)	Th2 cytokine regulation; eosinophil recruitment	Drives eosinophilic inflammation by suppressing PU.1; facilitates production of eotaxin, periostin, and IL-4/IL-5/IL-13	Murine genetic KO (allergen model); human case-control biomarker studies	[12, 19, 20]
miR-146a	Context-dependent: upregulated in allergic asthma; induced by rhinovirus; genetic deficiency aggravates antiviral responses	Human bronchial epithelial cells; plasma (pediatric human); murine OVA model	TRAF6; IRAK1 (NF-κB pathway transducers)	NF-κB inhibition; glucocorticoid augmentation; interferon-λ induction (viral context)	Negative feedback on NF-κB; augments steroid anti-inflammatory effects in allergic asthma; context-dependent: may impair antiviral immunity when deficient	Human cell in-vitro; murine in-vivo pharmacological mimic study; human plasma biomarker cohort	[22, 23, 24, 25]
let-7 family (let-7a, let-7c, let-7f)	Downregulated	Bronchial biopsies (severe asthma, human); plasma (pediatric human); circulating exosomes (longitudinal rhinovirus challenge)	HMGA2 (predicted); IL-13 pathway regulators (proposed)	Epithelial homeostasis; Th2 pathway inhibition	Maintains epithelial homeostasis; opposes Th2-permissive gene expression; downregulation associated with eosinophilia and impaired lung function; dynamically altered during viral exacerbation	Human biopsy sequencing; human plasma biomarker studies; longitudinal exosomal profiling	[27, 28, 30, 31]
miR-511-3p	Downregulated	Murine allergic asthma; miR-511-3p KO mice; lung macrophages	Complement component C3 (validated in EV delivery model)	Complement pathway; macrophage polarization	Restricts complement-mediated macrophage activation; loss promotes allergic inflammation; mannose-EV	Murine genetic KO; in-vivo EV therapeutic delivery model	[13]

					delivery reverses murine allergic airway inflammation		

Abbreviations: ASM, airway smooth muscle; ASMC, airway smooth-muscle cell; EV, extracellular vesicle; HDAC2, histone deacetylase 2; IRAK1, IL-1 receptor-associated kinase 1; KO, knockout; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; OVA, ovalbumin; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; TRAF6, tumor necrosis factor receptor-associated factor 6.

Table 2. Circulating and Extracellular-Vesicle miRNAs Evaluated as Asthma Biomarkers

miRNA(s)	Sample type	Population (age group)	Cohort size	Assay platform	Diagnostic or clinical metric	Validation status	Main limitation(s)	Reference
miR-21, miR-155	Serum	Adults (asthmatic vs. healthy controls)	40 asthmatic; 20 controls	qRT-PCR	miR-21: AUC not explicitly reported; sensitivity 100%, specificity 95% at cut-off 1.37. miR-155: sensitivity 100%, specificity 100% at cut-off 1.96. [Exploratory; not validated]	Discovery cohort only; no external validation	Very small case-control; healthy control comparator inflates performance vs. clinical scenario; no confidence intervals reported	[19]
Exosomal miR-155, miR-221	Serum exosomes	Adults; moderate-to-severe asthma vs. healthy controls	18 asthmatic; 18 controls	qRT-PCR	miR-155 AUC = 0.91; miR-221 AUC = 0.76. miR-221 positively correlated with FVC	Single discovery cohort; no external validation	Very small cohort; MISEV2018 exosome characterization extent unclear; no confidence intervals; no comparator respiratory disease group	[20]
miR-21, miR-146a	Plasma	Children (asthmatic vs. healthy controls)	Not fully reported	qRT-PCR	Significant upregulation in asthmatic children; positive correlations with IL-13 and eosinophil counts. No AUC reported	Discovery cohort only	No formal diagnostic performance metric (AUC/sensitivity/ specificity) reported; cohort size details incomplete	[25]
miR-155, let-7a	Plasma	Children (asthmatic vs. healthy controls; severity subgroups)	Not fully reported	qRT-PCR + ROC analysis	ROC analysis performed; differential expression by severity. Specific AUC/sensitivity/ specificity details not available in reviewed literature	Discovery cohort only	Performance metrics not fully extractable from available sources; pediatric-specific; no external validation	[30]
Exosomal miRNA panel (27	Plasma exosomes; saliva	Children stratified by lung function (severe	Not fully reported	Small RNA sequencing	27 differentially expressed plasma exosomal miRNAs; 40 salivary. Pathway	Discovery cohort only	No AUC for individual miRNAs; cohort size and phenotype details require	[36]

plasma; 40 salivary, including miR-21-related targets)	exosomes	obstruction vs. normal)			enrichment: T-cell and B-cell signaling. No individual AUC values reported		confirmation from the original publication	
Exosomal miRNA panel (26 miRNAs including let-7a, let-7f, miR-126-3p)	Plasma exosomes	Mild asthmatic adults and healthy controls; experimental rhinovirus challenge (longitudinal)	Not fully reported	Small RNA sequencing (longitudinal)	26 differentially expressed exosomal miRNAs; temporal patterns correlated with Th1/interferon and Th2/inflammatory signatures during viral challenge	Longitudinal case-control; no independent validation	No diagnostic AUC; experimental viral challenge may not replicate natural exacerbations; cohort size requires confirmation	[31]
miR-126a, miR-146a	Serum	Middle-aged and elderly adults during exacerbation vs. follow-up (stable)	n = 17	qRT-PCR	No AUC; elevated during exacerbation; positive correlation with Asthma Control Test results	Discovery cohort only	Very small cohort (n = 17); correlations with clinical outcomes weak; limited diagnostic utility; no between-subject comparator group described	[26]