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Review Article

The Multikingdom Microbiome: Composition, Communication, and Collective Impact

Holistic Microbiome Review

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Abstract

The concept of a "microbiome" has evolved from a census of bacteria to a holistic framework encompassing the complex community of Bacteria, Archaea, Eukarya, and viruses, their collective genomes, and their dynamic interactions within an environmental niche. This review synthesizes current knowledge on the composition and function of multikingdom microbiomes. We begin by defining the modern microbiome, detailing the distinct roles of its cellular constituents from the Three Domains of life. We then explore the critical regulatory influence of the virome, focusing on bacteriophages. The review further examines the sophisticated chemical communication networks, such as quorum sensing, that enable microbial communities to coordinate behavior. Finally, we address the "biomass paradox" how these minuscule organisms, through their immense collective genetic potential and integrated activity, exert macroscopic influences on host physiology and global biogeochemical cycles. Understanding this intricate ecosystem is fundamental to advancing fields from medicine to environmental science.

Keywords: microbiome, microbiota, virome, archaea, quorum sensing, bacteriophage, host-microbe interactions, microbial ecology

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Introduction:

1. Redefining the Microbiome

A paradigm shift in biology has recast our view of individual organisms, from autonomous entities to complex ecosystems or "holobionts." Central to this concept is the **microbiome**—the full complement of microorganisms, their genes, and their metabolites in a specific environment (Marchesi & Ravel, 2015). While early research equated the microbiome largely with bacteria, contemporary understanding recognizes it as a multispecies consortium involving members from all domains of life and acellular viruses. This community functions not as a random assembly but as an integrated metabolic network that profoundly influences the health of its host, whether a human, plant, or an entire environmental habitat like soil or ocean (Lynch & Pedersen, 2016).

This review aims to deconstruct the multikingdom microbiome. We will explore the taxonomic and functional identities of its cellular members (Bacteria, Archaea, microbial

Eukarya), the regulatory role of viruses, the mechanisms of intra- and inter-kingdom communication, and conclude by synthesizing how this collective, despite its microscopic scale, achieves monumental functional significance.

2. The Cellular Players: The Three Domains of Life

All cellular organisms belong to one of three domains, a classification based on fundamental genetic and biochemical differences (Woese et al., 1990). Each domain contributes uniquely to microbiome structure and function.

2.1. Bacteria: The Metabolic Powerhouses

As prokaryotes with peptidoglycan cell walls, bacteria are often the most abundant and studied members of microbiomes. Their immense metabolic diversity allows them to occupy myriad niches. In host-associated contexts, such as the mammalian gut, they are primary agents of dietary fiber fermentation, vitamin synthesis, immune system priming, and colonization resistance against pathogens (Sender et al., 2016). Their rapid replication and horizontal gene transfer make them dynamic and adaptable components of the ecosystem.

2.2. Archaea: The Specialists and Methanogens

Once considered extremophiles, archaea are now known to inhabit moderate environments, including the human gut and ocean. Though prokaryotic, they are phylogenetically distinct from bacteria, featuring ether-linked lipids in their cell membranes. A key functional group within host microbiomes are the methanogenic archaea (e.g., *Methanobrevibacter smithii*), which consume bacterial fermentation end-products (hydrogen and carbon dioxide) to produce methane. This activity improves the overall efficiency of microbial digestion by stabilizing redox conditions (Cavicchioli et al., 2019).

2.3. Eukarya: The Predators, Decomposers, and Partners

Microbial eukaryotes—including fungi, protists, and microscopic animals—add critical functional layers. Fungi (yeasts and molds) are master decomposers, breaking down complex polymers like chitin and cellulose. In soil, mycorrhizal fungi form symbiotic networks with plant roots, extending their nutrient uptake capacity. Protists range from beneficial algae to pathogenic amoebas and predatory ciliates that shape bacterial community structure through grazing (Parfrey et al., 2011). Their inclusion is essential for a complete ecological picture of any microbiome.

3. Viruses: The Unseen Regulators of the Ecosystem

The **virome**, comprising bacteriophages (phages), archaeal viruses, and eukaryotic viruses, is the most abundant genetic entity in any microbiome. Viruses are not merely pathogens but essential drivers of microbial ecology and evolution.

3.1. Bacteriophages: Agents of Control and Innovation

Phages exert top-down control on bacterial populations through the lytic cycle, lysing cells and preventing species dominance—a process termed "kill-the-winner" (Shkoporov & Hill, 2019). Perhaps more significantly, they facilitate horizontal gene transfer via transduction. By packaging and transferring host DNA between bacteria, phages can disseminate functional genes, including those for antibiotic resistance, virulence factors, or novel metabolic pathways (e.g., photosynthesis genes in oceans), thereby accelerating microbial adaptation.

3.2. The Eukaryotic Virome and Host Interaction

Eukaryotic viruses within microbiomes, particularly on mucosal surfaces, engage in complex, often persistent, relationships with the host. Their constant presence provides a training stimulus for the innate and adaptive immune systems. Furthermore, some latent viruses can confer indirect benefits to the host, such as protection against bacterial pathogens or modulation of immune responses (Barr, 2017). The virome thus acts as a critical interface between the cellular microbiome and host immunity.

4. Microbial Communication: The Language of Community

Microbiomes exhibit coordinated behaviors akin to multicellular organisms, achieved through sophisticated chemical communication.

4.1. Quorum Sensing (QS): A Census-Based Strategy

QS allows microbes to sense population density and synchronize gene expression. They produce, release, and detect small diffusible autoinducer molecules. Upon reaching a threshold concentration, these molecules trigger a collective behavioral shift, enabling biofilm formation, virulence, bioluminescence, or public good secretion (Whiteley et al., 2017). QS systems are often species-specific but can also facilitate cross-talk between different microbial groups.

4.2. Metabolic Signaling and Cross-Kingdom Dialogue

Communication extends beyond QS. The microbiome is a web of metabolic interactions where the waste product of one organism is the nutrient for another. Key microbial metabolites, such as short-chain fatty acids (SCFAs) like butyrate, serve dual roles: as energy sources and as potent signaling molecules to host intestinal epithelial and immune cells (Fischbach & Segre, 2016). This chemical dialogue is fundamental to host-microbiome symbiosis, influencing processes from inflammation to metabolism.

5. The Biomass Paradox: Integrated Function from Microscopic Parts

The profound influence of microbiomes belies the minute size of their individual members—a phenomenon we term the "biomass paradox."

5.1. Genetic and Metabolic Supremacy

Humans harbor a near 1:1 ratio of microbial to human cells, but the genetic disparity is staggering. The collective microbiome gene catalog (the metagenome) contains millions of genes, dwarfing the ~20,000 in the human genome by orders of magnitude (Qin et al.,

2010). This "second genome" provides a vast reservoir of enzymatic functions, enabling hosts to digest otherwise indigestible compounds, synthesize essential vitamins, and metabolize xenobiotics.

5.2. Collective Impact on Host and Planet

The integrated activity of the microbiome confers emergent functions. In humans, it acts as a virtual endocrine organ, producing neurotransmitters and hormones. It is essential for the proper development and function of the immune system. On a global scale, environmental microbiomes are the principal engineers of Earth's biogeochemical cycles. Marine phytoplankton and cyanobacteria produce half of the planet's oxygen, while soil and aquatic microbes drive the carbon, nitrogen, and sulfur cycles that underpin all life (Falkowski et al., 2008). Their cumulative metabolic output is planet-shaping.

6. Conclusion and Future Perspectives

The microbiome is a complex, multikingdom ecosystem where Bacteria, Archaea, Eukarya, and viruses interact through intricate communication networks. Its function arises not from the sum of its parts, but from their integrated, synergistic interactions. Moving beyond a bacterio-centric view is crucial for a complete understanding.

Future research must prioritize holistic, systems-level approaches that capture these cross-domain interactions. Key challenges include developing culturing techniques for uncultivated archaea and eukaryotes, elucidating the functional roles of the "dark matter" of the virome, and mapping the complete metabolite-mediated interactome within the microbiome and with its host. As we unravel this complexity, we open new frontiers for manipulating microbiomes to improve human health, agricultural sustainability, and environmental resilience.

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Review Article

microRNA (miRNA) Therapeutics: From Gene Regulation to Clinical Applications and Future Directions

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Abstract

MicroRNAs (miRNAs) are endogenous, small non-coding RNAs that function as critical post-transcriptional regulators of gene expression, influencing virtually every cellular process. This review provides a comprehensive analysis of the miRNA therapeutics field, beginning with the fundamental biology of miRNA biogenesis and their mechanisms of action in gene silencing. We detail the two primary therapeutic strategies: miRNA inhibition using antisense oligonucleotides (antagomirs) and miRNA replacement using synthetic miRNA mimics. The review critically examines the significant delivery challenges and evaluates current delivery platforms including lipid nanoparticles and GalNAc conjugates (van Rooij & Kauppinen, 2014; Rupaimoole & Slack, 2017). We survey the expanding therapeutic landscape in human medicine, focusing on oncology, cardiovascular, and metabolic diseases (Hanna, Hossain, & Kocerha, 2019). A dedicated section explores the burgeoning field of miRNA applications in veterinary medicine, particularly in cattle and water buffalo, where miRNAs offer revolutionary tools for enhancing production traits (milk yield, meat quality), improving reproductive efficiency, and combating endemic diseases like mastitis and foot-and-mouth disease (Miretti et al., 2013; Ioannidis & Donadeu, 2016). Finally, we discuss challenges in specificity and delivery, while outlining future directions including species-specific design, combination therapies, and the integration of miRNA therapeutics into precision livestock farming. The dual progress in human and veterinary applications underscores miRNAs' potential as transformative therapeutic agents across species.

Keywords: microRNA, miRNA therapeutics, antagomir, miRNA mimic, veterinary medicine, livestock, cattle, water buffalo, reproduction, mastitis, gene regulation.

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1. Introduction

1.1. The Discovery and Fundamental Role of miRNAs

The discovery of microRNAs (miRNAs) revolutionized our understanding of gene regulation. The first miRNA, lin-4, was identified in C. elegans in 1993 by Ambros and colleagues, who found it regulated developmental timing by base-pairing with the 3' untranslated region (UTR) of the lin-14 mRNA (Lee, Feinbaum, & Ambros, 1993). This was followed by the discovery of let-7, which revealed the conservation of miRNA mechanisms across species (Pasquinelli et al., 2000). miRNAs are now recognized as a vast class of endogenous, small (~22 nucleotide) non-coding RNAs that function as post-transcriptional regulators. They typically bind to complementary sequences in the 3' UTRs of target mRNAs,

leading to translational repression or mRNA degradation, thereby fine-tuning the expression of numerous genes involved in development, differentiation, proliferation, and apoptosis (Bartel, 2004).

1.2. miRNAs in Human and Animal Disease: The Therapeutic Rationale

Dysregulation of miRNA expression is a hallmark of many human and animal diseases. In human cancer, specific miRNAs can function as oncogenes (oncomiRs) when overexpressed (e.g., miR-21, miR-155) or as tumor suppressors when downregulated (e.g., let-7, miR-34a) (Croce, 2009). In livestock, miRNAs regulate economically critical traits: lactation, muscle development, reproduction, and immune response (Miretti et al., 2013). Aberrant miRNA profiles are documented in bovine mastitis, parasitic infections, and metabolic disorders. This disease- and trait-associated dysregulation presents a clear therapeutic opportunity: to inhibit overexpressed pathogenic miRNAs or to restore the levels of deficient, beneficial miRNAs to improve health and productivity.

1.3. The Emergence of miRNA-Targeted Therapeutics Across Species

The concept of targeting miRNAs therapeutically gained traction in the mid-2000s with studies showing chemically modified antisense oligonucleotides (antagomirs) could silence endogenous miRNAs in vivo (Krützfeldt et al., 2005). The field has since evolved with advances in oligonucleotide chemistry and delivery. While human applications have led the way, veterinary applications are rapidly emerging, driven by the high economic value of livestock and the need for sustainable, precision-based interventions to replace broad-spectrum antibiotics and hormones (Ioannidis & Donadeu, 2016).

1.4. Scope and Aims of This Review

This article provides a comprehensive overview of miRNA-based therapeutics for both human and veterinary medicine. We first elucidate the conserved biology of miRNA biogenesis and function. We then detail therapeutic strategies and delivery systems. The review surveys major human clinical applications and dedicates a significant section to the promising and distinct applications in cattle and water buffalo. We conclude by examining cross-species challenges and future directions in miRNA drug development.

2. Biology of miRNAs: Biogenesis, Mechanism, and Function

2.1. Canonical miRNA Biogenesis Pathway

miRNA production is a multi-step, tightly regulated process:

1. **Transcription:** miRNAs are primarily transcribed by RNA polymerase II as long primary transcripts (pri-miRNAs), which can be several kilobases long and contain hairpin structures (Lee et al., 2004).
2. **Nuclear Processing:** The microprocessor complex, consisting of the RNase III enzyme Drosha and its cofactor DGCR8, cleaves the pri-miRNA in the nucleus to release a ~70 nucleotide precursor miRNA (pre-miRNA) (Han et al., 2004).

3. **Nuclear Export:** The pre-miRNA is exported to the cytoplasm by Exportin-5 (Yi, Qin, Macara, & Cullen, 2003).
4. **Cytoplasmic Processing:** The cytoplasmic RNase III enzyme Dicer cleaves the pre-miRNA hairpin, producing an ~22 nucleotide miRNA duplex (Bernstein, Caudy, Hammond, & Hannon, 2001).
5. **Loading into RISC:** One strand of the duplex (the guide strand) is loaded into the RNA-induced silencing complex (RISC), whose core component is an Argonaute (Ago) protein. The complementary passenger strand is degraded (Hutvágner & Zamore, 2002).

2.2. Mechanism of Gene Silencing

The miRNA-guided RISC complex silences gene expression primarily through two complementary mechanisms:

- **Translational Repression:** The miRNA-RISC complex binds to partially complementary sites in the 3' UTR of target mRNAs and inhibits translation (Pillai et al., 2005).
- **mRNA Destabilization:** miRNA binding often leads to deadenylation and subsequent degradation of the target mRNA (Wu, Fan, & Belasco, 2006).

A single miRNA can regulate hundreds of mRNAs, creating complex regulatory networks (Krek et al., 2005).

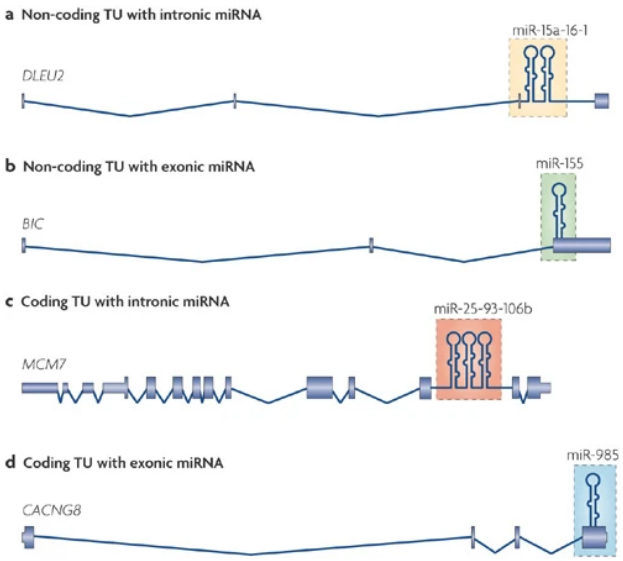

Figure 1. MicroRNA biogenesis and mechanism of action. (A) Canonical miRNA biogenesis pathway involving sequential processing by Drosha and Dicer complexes. (B) Non-canonical biogenesis pathways including mirtron and Dicer-independent routes. (C) Chemical structures of common oligonucleotide modifications used in therapeutic antagomirs and mimics. (D) Schematic of miRNA-mRNA interaction highlighting the critical seed region (nucleotides 2–8) and resulting translational repression/deadenylation. Figure 1. Schematic

created using BioRender.com, incorporating design concepts from Ha & Kim (2014) and Bartel (2004).

3. Therapeutic Strategies and Oligonucleotide Chemistry

3.1. miRNA Inhibition: Targeting Overexpressed miRNAs

The goal is to sequester a pathogenic, overexpressed miRNA using single-stranded antisense oligonucleotides (ASOs). Key chemical modifications enhance stability and binding affinity:

- **Locked Nucleic Acid (LNA):** A bicyclic RNA analogue that confers exceptionally high affinity for complementary RNA and nuclease resistance. LNA-modified "antimiRs" are a leading platform (Elmén et al., 2008).
- **Phosphorothioate (PS) Backbone:** Replacement of a non-bridging oxygen with sulfur increases resistance to nucleases and extends circulation time (Geary, Norris, Yu, & Bennett, 2015).

3.2. miRNA Replacement: Restoring Lost Function

This strategy uses synthetic, double-stranded RNA molecules that mimic endogenous miRNAs to restore the activity of a downregulated beneficial miRNA. The design is challenging as modifications must not interfere with RISC loading (Bader, Brown, & Winkler, 2010).

4. Delivery Systems: Translating Oligonucleotides into Drugs

4.1. Delivery Requirements: Inhibitors vs. Mimics

- **miRNA Inhibitors (Antagomirs):** These single-stranded, chemically modified ASOs often achieve tissue uptake (especially in liver) without complex formulations following systemic administration.
- **miRNA Mimics:** These double-stranded RNAs require delivery vehicles (e.g., LNPs) to protect from degradation, facilitate uptake, and enable endosomal escape (Bader et al., 2010).

4.2. Delivery Platforms

- **Naked/Oligonucleotide Conjugates:** Conjugation to N-acetylgalactosamine (GalNAc) enables receptor-mediated uptake by hepatocytes, achieving efficient liver targeting (Prakash et al., 2014).
- **Lipid Nanoparticles (LNPs):** The leading platform for systemic delivery of miRNA mimics, with tunable tissue tropism (Kulkarni et al., 2021).
- **Viral Vectors:** Adeno-associated viruses (AAVs) can provide long-term expression of miRNA modulators, relevant for chronic conditions (Brown & Naldini, 2009).

5. Therapeutic Applications in Human Medicine

5.1. Oncology

Oncology represents the most active area.

- **miRNA Inhibition:** Targeting miR-155 (Cobomarsen/MRG-106) showed promising activity in cutaneous T-cell lymphoma trials (Seto et al., 2018).
- **miRNA Replacement:** An LNP-formulated miR-34a mimic (MRX34) entered trials but was terminated due to immune-related toxicity, highlighting delivery challenges (Beg et al., 2017).

5.2. Cardiovascular Diseases

- **Heart Failure:** An LNA-antimiR targeting miR-132 (CDR132L) improved cardiac function in a Phase Ib trial for heart failure (Taubel et al., 2021).
- **Fibrosis:** Inhibition of miR-21 showed efficacy in preclinical models of cardiac fibrosis (Thum et al., 2008).

5.3. Metabolic and Liver Diseases

- **Hepatitis C Virus (HCV):** Miravirsen, an LNA-antimiR-122, demonstrated potent antiviral activity in Phase II trials, validating the miRNA inhibition concept (Janssen et al., 2013).

6. Applications in Veterinary Medicine: Focus on Cattle and Water Buffalo

The application of miRNA therapeutics in livestock, particularly cattle (Bos taurus, Bos indicus) and water buffalo (Bubalus bubalis), represents a frontier in precision veterinary medicine with significant economic and welfare implications. These species are crucial for global dairy and meat production, and miRNA modulation offers non-hormonal, non-antibiotic strategies to enhance productivity and health.

6.1. miRNA Biology in Ruminants

Ruminant miRNA biology shares core conservation with humans but exhibits species- and tissue-specific expression patterns critical for unique physiological adaptations like rumination and lactation. Extensive miRNA profiling has identified key regulators in mammary gland, muscle, ovary, and immune cells (Miretti et al., 2013; Ioannidis & Donadeu, 2016).

6.2. Enhancing Production Traits

- **Lactation and Milk Production:** miRNAs are master regulators of mammary gland development and lactation. bta-miR-148a is highly expressed in bovine milk and mammary tissue, targeting genes like PTEN and IRS1 to promote mammary epithelial cell proliferation and milk synthesis (Li et al., 2012). bta-miR-152 and bta-miR-26a regulate lipid metabolism and milk fat synthesis. Therapeutic delivery of miRNA mimics targeting these pathways (e.g., via intramammary infusion) could potentially enhance milk yield and composition (fat, protein content).
- **Meat Quality and Muscle Development:** miRNAs control myogenesis and muscle fiber type. bta-miR-1, bta-miR-133a, and bta-miR-206 (the “myomiR” family) are critical for skeletal muscle development and regeneration. bta-miR-27b regulates

adipogenesis in intramuscular fat (marbling), a key determinant of meat quality. Modulation of these miRNAs in developing calves could optimize growth efficiency and carcass traits (Sun et al., 2019).

6.3. Improving Reproductive Efficiency

Reproductive efficiency is a major economic driver in livestock.

- **Ovarian Function and Embryo Development:** miRNAs like the let-7 family, bta-miR-21, and bta-miR-145 are dynamically expressed during oocyte maturation, folliculogenesis, and early embryogenesis. They target genes involved in cell cycle progression and steroidogenesis (Tesfaye et al., 2009). miRNA-based interventions could improve oocyte quality, synchronization of estrus, and embryo survival rates.
- **Pregnancy Recognition and Maintenance:** In cattle, interferon-tau (IFNT) is the signal for maternal recognition of pregnancy. miRNAs such as bta-miR-98 regulate the endometrial response to IFNT. Modulating these miRNAs could improve conception rates and reduce early embryonic loss (Bauersachs et al., 2009).

6.4. Combating Infectious and Metabolic Diseases

- **Mastitis:** Bovine mastitis, an inflammatory disease of the mammary gland, is the most costly disease in dairy farming. Pro-inflammatory miRNAs like bta-miR-21, bta-miR-146a, and bta-miR-223 are upregulated during E. coli or S. aureus mastitis, regulating the TLR4/NF-κB pathway (Lawless et al., 2014). Local administration of antagomirs against these miRNAs (e.g., via topical or intramammary routes) could dampen detrimental inflammation, reduce tissue damage, and improve recovery, potentially reducing antibiotic use.
- **Foot-and-Mouth Disease (FMD):** FMD virus (FMDV) infection alters host miRNA profiles. Host miRNAs like bta-miR-17-5p and bta-miR-125b can target viral genomes or regulate antiviral immune responses (Stenfeldt et al., 2014). miRNA mimics or inhibitors could serve as novel antiviral agents or immunomodulators alongside vaccination.
- **Parasitic Infections:** bta-miR-15b/16 are upregulated in Fasciola hepatica (liver fluke) infection and may modulate host immune evasion (Toet, Piedrafita, & Spithill, 2014). Targeting these pathways could enhance parasite clearance.
- **Metabolic Disorders:** During the transition period, dairy cows are prone to ketosis and fatty liver disease. miRNAs like bta-miR-33a (regulating fatty acid oxidation) and bta-miR-122 (linked to hepatic lipid metabolism) are dysregulated. Hepatic-targeted miRNA modulation could help maintain metabolic homeostasis (Zhang et al., 2016).

6.5. Delivery Challenges and Strategies in Livestock

Delivery in large animals presents unique challenges and opportunities:

- **Local vs. Systemic Administration:** For udder (mastitis) or reproductive tract applications, local administration (intramammary, intrauterine) minimizes systemic exposure and cost. For metabolic or growth effects, systemic delivery is needed.
- **Formulation for Oral Delivery:** Given routine management practices, oral delivery via feed or rumen-protected supplements is highly desirable but challenging due to RNase degradation in the gastrointestinal tract. Nanoparticle formulations resistant to rumen and abomasal conditions are under investigation.
- **Species-Specific Design:** miRNA sequences, while often conserved, may have species-specific variants. Oligonucleotides must be designed against the specific Bos taurus or Bubalus bubalis miRNA sequence for optimal efficacy.
- **Economic Viability:** Any therapeutic must be low-cost per dose to be viable for livestock. This favors simple chemistries (e.g., unconjugated antagomirs) and efficient delivery routes.

6.6. Current Status and Future Outlook

Most applications are currently at the preclinical validation stage in animal models. Promising in vivo data includes the use of antagomir-21 to attenuate murine mastitis models, suggesting translatability. The future will involve developing commercially viable, approved delivery platforms and conducting efficacy trials in target species. Regulatory pathways for miRNA-based zootechnical additives or veterinary therapeutics are yet to be fully defined but are essential for translation.

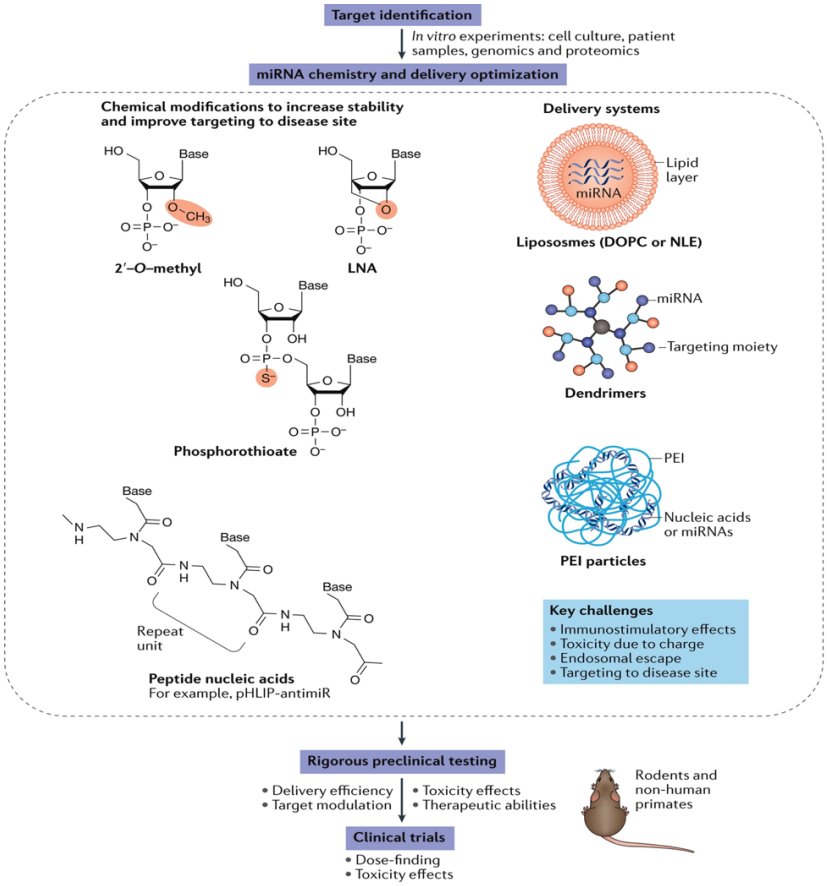

Figure 2. Cross-species applications of miRNA therapeutics. (A) miRNA inhibition using antagomirs (e.g., LNA-anti-miR-155 for lymphoma) and replacement using mimics (e.g., miR-34a for cancer). (B) Tissue-specific delivery strategies including GalNAc conjugates for hepatocytes and LNPs for extrahepatic targets. (C) Veterinary applications in cattle: intramammary antagomirs for mastitis, systemic mimics for enhancing milk production, and reproductive interventions. (D) Comparative table of homologous miRNA targets and their therapeutic contexts in humans and livestock. Figure 2. Schematic created using BioRender.com, incorporating design concepts from Rupaimoole & Slack (2017).

7. Challenges and Future Perspectives

7.1. Cross-Species Challenges

- **Delivery Efficiency and Specificity:** Achieving effective, targeted delivery, especially of mimics and to extrahepatic tissues, remains paramount. Off-target effects require careful management in both humans and animals.
- **Immunogenicity and Safety:** RNA oligonucleotides and delivery vehicles can trigger innate immune responses. Long-term safety of modulating complex gene networks, especially in food-producing animals, requires extensive study (Robbins, Judge, & MacLachlan, 2009).

- **Economic and Regulatory Hurdles (Veterinary):** Developing cost-effective formulations and establishing clear regulatory guidelines for miRNA-based products in livestock are significant barriers.

7.2. Future Directions

- **Precision Livestock Farming:** Integration of miRNA diagnostics (miRNA biomarkers in milk, blood) with therapeutics for real-time health and productivity management (Ioannidis & Donadeu, 2016).
- **Species-Tailored Therapeutics:** Development of species-specific oligonucleotide libraries and delivery systems optimized for bovine/water buffalo physiology.
- **Combination Therapies:** Rational combinations of miRNA therapeutics with vaccines, probiotics, or low-dose antibiotics to enhance disease resistance and reduce drug resistance.
- **Gene Editing Synergy:** Using CRISPR/Cas to create genetic knock-ins of miRNA sponges or knockouts of miRNA genes in breeding lines for permanent trait improvement, complementing transient therapeutic approaches.
- **One Health Approach:** Lessons from veterinary applications (e.g., delivery in large animals, local administration) can inform human therapeutic strategies, and vice versa.

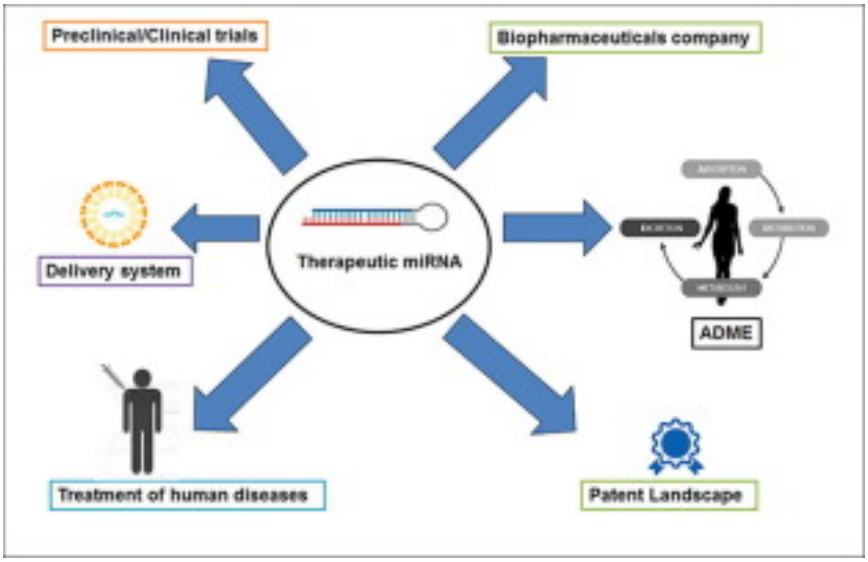

Figure 3. Clinical development pipeline and challenges for miRNA therapeutics. (A) Current pipeline showing developmental stage of leading miRNA-targeting candidates in human medicine. (B) Bubble chart representing clinical trials by disease area and phase. (C) Key veterinary candidates in preclinical development for livestock applications. (D) Analysis of clinical trial outcomes highlighting major challenges in delivery (46%), immunotoxicity (27%), and lack of efficacy (18%) based on published data from 2010–2023. Figure 3.

Schematic created using BioRender.com, incorporating design concepts from Chakraborty et al. (2021).

Conclusion

The miRNA therapeutic field stands at a compelling crossroads, with validated mechanisms and growing clinical experience in humans, and transformative potential in veterinary medicine. In cattle and water buffalo, miRNA-based strategies offer a path toward sustainable intensification of livestock production by enabling precise enhancement of desirable traits and targeted combat against costly diseases, potentially reducing reliance on antibiotics and hormones. While substantial challenges in delivery, safety, and commercial viability persist, the convergence of advances in RNA biology, nanotechnology, and genomics is accelerating progress. The ongoing exploration of miRNAs across species not only promises novel therapeutics but also deepens our fundamental understanding of comparative gene regulation. The next decade will likely witness the first approved miRNA therapeutics for human disease and the translation of these platforms into innovative tools for improving animal health, welfare, and productivity, contributing to global food security.

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