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RESEARCH ARTICLE

SIRNA: ITS TRANSLATION FROM RESEARCH TO THERAPEUTIC APPLICATIONS

Prerana Prakash and M G Hariprasad

1. KLE College of Pharmacy, Rajajinagar, II Block, Bengaluru-560010. KLE Academy of Higher Education and Research, Belagavi, 590010

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Abstract

Small interfering RNA (siRNA) is a short, double-stranded RNA molecule that has emerged as a pivotal tool for gene silencing through the RNA interference (RNAi) pathway. It is a powerful post-transcriptional gene-silencing molecule central to RNA interference (RNAi) mechanisms¹. Typically 21–25 nucleotides in length, it guides the RNA-induced silencing complex (RISC) to a target messenger RNA (mRNA), enabling sequence-specific degradation and effectively silencing gene expression². This process is highly specific and forms the foundation for siRNA's role in research and therapeutic interventions³. By guiding the RNA-induced silencing complex (RISC) to target mRNA, siRNA enables post-transcriptional gene regulation with unparalleled precision. This targeted action holds immense promise in treating genetic, infectious, and degenerative diseases⁴. Therapeutically, siRNA offers unique advantages over traditional treatments, including the ability to target undruggable proteins, rapid development timelines, and reduced systemic toxicity. These advantages have catalyzed interest in siRNA for precision medicine and rare genetic disorders⁵.

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Introduction:-

History and Discovery

The siRNA journey began in 1990 when gene silencing was first reported in plants (petunias). This phenomenon, termed "co-suppression," hinted at a broader biological principle⁶. The landmark discovery came in 1998 when Andrew Fire and Craig Mello demonstrated that injecting double-stranded RNA into *C. elegans* could silence specific genes — a mechanism later known as RNA interference⁷. This breakthrough revolutionized molecular genetics, and earned them the Nobel Prize in 2006. By 2001, Tuschl and colleagues synthesized siRNA and used it to silence genes in mammalian cells, marking a significant milestone in therapeutic development. This paved the way for siRNA's translation from experimental research to clinical innovation⁸.

The discovery of small interfering RNA (siRNA) marked a paradigm shift in the understanding of gene regulation. The roots of this breakthrough lie in observations from the early 1990s, where researchers noted unexpected suppression of transgenes in plants—termed post-transcriptional gene silencing (PTGS)⁹. Similar mechanisms were later identified in fungi and animals, suggesting a conserved biological process¹⁰. The key turning point came in 1998, when Andrew Fire and Craig Mello demonstrated that introducing double-stranded RNA (dsRNA) into

Caenorhabditis elegans could silence specific genes far more effectively than single-stranded RNA. Their work, which earned the 2006 Nobel Prize in Physiology or Medicine, confirmed that dsRNA triggered a potent and specific gene-silencing mechanism now known as RNA interference (RNAi)¹¹.

Subsequent research revealed that long dsRNA is processed by the enzyme Dicer into 21–23 nucleotide fragments, termed siRNAs. These siRNAs are incorporated into the RNA-induced silencing complex (RISC), which uses one strand as a guide to recognize and cleave complementary messenger RNA (mRNA), thereby suppressing gene expression. This mechanism, initially thought to be exclusive to lower organisms, was soon confirmed to operate in mammalian cells under certain conditions¹².

siRNAs belong to a broader family of small RNAs that includes microRNAs (miRNAs) and piwi-interacting RNAs (piRNAs). While siRNAs typically originate from exogenous or synthetic sources and show perfect complementarity to their mRNA targets, miRNAs are endogenously encoded and often act through partial pairing to suppress translation¹³. In contrast, piRNAs function in germline cells to silence transposable elements and operate via Dicer-independent mechanisms¹⁴.

The discovery of siRNA had profound implications for biomedical science. It provided a precise and reversible method to silence genes, which transformed functional genomics research¹⁵. Almost immediately, siRNAs were explored as potential therapeutics for a variety of genetic and infectious diseases. However, the translation from bench to bedside was not without hurdles—issues such as delivery efficiency, stability in vivo, off-target effects, and immune responses initially hampered clinical development¹⁶.

Nonetheless, the foundational discovery of siRNA remains a landmark achievement in molecular biology, laying the groundwork for the current and future era of RNA-based therapeutics¹⁷.

Research Applications of siRNA

In biomedical research, siRNA serves as a critical tool for functional genomics by enabling targeted gene knockdown. Scientists have employed siRNA to:

- Study cancer biology by silencing oncogenes or tumor suppressors.
- Model neurodegenerative diseases such as Alzheimer's and Parkinson's.
- Validate drug targets and understand disease mechanisms¹⁸.

In infectious disease research, siRNA has been used to dissect host–pathogen interactions by selectively silencing host genes involved in viral entry, replication, or immune evasion—contributing significantly to antiviral drug development. For instance, siRNAs targeting host factors essential for HIV or influenza virus replication have identified novel therapeutic targets¹⁹.

Additionally, in cardiovascular research, siRNA has been employed to study genes involved in lipid metabolism, atherosclerosis, and hypertension. Its ability to downregulate disease-associated genes in animal models has enabled the preclinical evaluation of gene-targeted therapies².

Collectively, siRNA continues to be a cornerstone of translational research, bridging basic molecular biology and therapeutic innovation. Its versatility and effectiveness in modeling diseases and validating targets underscore its enduring value in the biomedical research landscape²¹.

It enables the systematic silencing of genes, aiding in the elucidation of gene function and the identification of disease-related pathways. siRNA has facilitated target validation, pathway dissection, and the modeling of genetic disorders in vitro and in vivo. It has been instrumental in studying cancer biology, virology, neurodegenerative diseases, and cardiovascular conditions. The simplicity of designing siRNAs against virtually any gene, coupled with their robust knockdown capabilities, underscores their utility in preclinical research²².

From Research to Therapeutic Applications

siRNA's transition from research to therapy materialized with the advent of FDA-approved drugs such as Patisiran (ONPATTRO®) (2018) for hereditary transthyretin-mediated amyloidosis and Givosiran (GIVLAARI™) (2019) for acute hepatic porphyria. These drugs demonstrated the clinical potential of siRNA in silencing harmful genes systemically with minimal off-target effects²³.

The advent of Inclisiran, an siRNA targeting PCSK9 to lower cholesterol, further exemplifies siRNA's therapeutic utility²⁴. Therapeutic siRNA must navigate biological barriers, remain stable in circulation, and target specific tissues. Advances in nanocarrier systems, including lipid nanoparticles (LNPs), have greatly enhanced siRNA delivery, enabling their therapeutic success in clinical trials and real-world use. Lipid nanoparticles (LNPs) and conjugate chemistries have enabled safe and effective delivery, making siRNA a cornerstone of modern therapeutics²⁵.

Table.1: siRNA delivery system and its therapeutic outcome²⁶

Sl.no	Disease	Target Genes	Delivery Systems/ Strategies	Therapeutic Outcomes
1	Cancer	KRAS, c-Myc, PD-L1	- Lipid nanoparticles - 2'-O-methylation - Advanced targeting modifications	- Tumor suppression - Immune activation - Reversal of drug resistance
2	Rheumatoid Arthritis	NF-κB p65, STAT1	- PEI-based nanocomplexes (Chen et al.) - RGD-modified PLGA NPs (Scheinman et al.)	- Reduced joint inflammation - Targeted macrophage/dendritic cell suppression
3	Ulcerative Colitis	NF-κB p65 and related cytokines	- MPEG-PCL- CH2R4H2C NPs (Ibaraki) - Silica-coated CaP NPs (Muller et al.)	- Suppressed inflammatory mediators - Improved clinical & histopathological scores in colitis models
4	Brain Inflammation	Inflammatory genes (e.g., post- ICH)	- Poly(β-amino ester) NPs - Receptor-mediated transcytosis - Intranasal/intracerebral delivery - LNPs, viral vectors	- Inflammation control - Effective BBB penetration - Neuroprotection and tumor inhibition

Limitations of siRNA

Despite the significant therapeutic potential of siRNA, several limitations must be addressed for its successful clinical application:

- **Instability in Biological Fluids:** Naked siRNA is highly susceptible to degradation by nucleases present in blood and tissues, limiting its half-life and therapeutic efficacy²⁷.
- **Rapid Renal Clearance:** Due to its small molecular size and hydrophilic nature, unmodified siRNA is quickly eliminated via the kidneys, reducing its systemic bioavailability²⁸.
- **Off-Target Effects:** siRNAs can inadvertently silence genes with partial sequence similarity, leading to unintended gene knockdown and potential toxicity²⁹.
- **Immune Stimulation:** Certain siRNA sequences may activate innate immune responses through Toll-like receptors (e.g., TLR3, TLR7/8), triggering inflammation or cytokine release³⁰.
- **Poor Cellular Uptake:** siRNA cannot easily cross cell membranes due to its negative charge and hydrophilicity, requiring specialized delivery systems to ensure intracellular access³¹.
- **Limited Tissue Specificity:** Achieving targeted delivery to specific tissues or cell types remains challenging, particularly in non-hepatic tissues³².
- **Risk of Saturating the RNAi Machinery:** High doses of exogenous siRNA may overload components of the endogenous RNAi pathway, potentially disrupting normal microRNA function³³.

Addressing these limitations necessitates the development of advanced delivery platforms (e.g., lipid nanoparticles, polymeric carriers) and chemical modifications (e.g., 2'-O-methyl, phosphorothioate linkages) to improve stability, specificity, and safety³⁴.

Approaches to overcome limitations of siRNA as a therapeutic agents

The clinical translation of siRNA has been significantly advanced through the development of innovative strategies aimed at overcoming its biological and pharmacokinetic limitations. These approaches span chemical engineering, delivery system design, and molecular optimization³⁵:

Table.2: Strategies to Enhance siRNA Therapeutics³⁶

Sl.no	Strategy	Description
1	Chemical Modifications to Enhance Stability and Reduce Immunogenicity	-2'-O-Methyl (2'-OMe)&2'-Fluoro (2'-F): Improve nuclease resistance and reduce immunostimulation. -LNA: Locked conformation enhances stability and binding. -Phosphorothioate Linkages: Improve plasma stability and degradation resistance. Note:-These modifications improve pharmacokinetics, reduce dosing frequency, and enhance safety profiles.
2	Advanced Delivery Systems	- Lipid Nanoparticles (LNPs): Prevent degradation and support cellular uptake. - Polymeric Carriers (e.g., PLGA, PEI): Controlled release and targeted delivery. - Peptide-Based Systems: Tissue-specific targeting via CPPs and ligands.
3	Hybrid & Multifunctional Platforms	- Combinatorial Systems: Integrate lipids, polymers, and targeting ligands for synergistic benefits. - Can include imaging agents for theranostics and monitoring therapeutic efficacy.
4	Sequence Optimization and Design	- Bioinformatics Tools: Aid in designing siRNAs with minimal off-target effects. - Guide Strand Modifications: Prevent unintended mRNA interactions and improve specificity.
5	siRNA Pooling Strategies	- Smart Pooling: Combines multiple siRNAs targeting different regions of the same gene. - Reduces off-target effects while maintaining silencing potency—useful in screening and early therapy stages.

Challenges in Development and Deployment of siRNA Therapeutics

Several challenges continue to hinder siRNA's widespread therapeutic use. Efficient and targeted delivery remains a bottleneck, especially for extrahepatic tissues. Formulating stable, biocompatible delivery vehicles that can navigate the immune system and reach target cells is critical³⁷. Additionally, large-scale manufacturing, cost, and regulatory hurdles affect clinical translation. Dosing regimens, long-term effects, and patient variability must be addressed through rigorous clinical studies. Despite these challenges, the expanding siRNA therapeutic pipeline reflects strong industry and academic interest in overcoming these barriers³⁸.

Table.3: Key Challenges at a Glance – siRNA Therapeutics³⁹

Sl.no	Challenges	Description
1	Targeting Beyond the Liver	siRNA therapies work well in the liver, but efficient delivery to organs like the brain, lungs, or joints remains a major hurdle.
2	Building the Perfect Carrier	Requires stable, biocompatible vehicles that evade the immune system, resist degradation, and accurately reach target cells.
3	Scale-Up Struggles	Large-scale production faces challenges in maintaining consistency, stability, and cost-efficiency for clinical deployment.
4	Cost and Complexity	High production and formulation costs limit affordability and accessibility, especially in low-resource healthcare settings.
5	Regulatory Roadblocks	Complex and evolving regulatory requirements demand robust safety and efficacy data, slowing clinical progress.
6	Dosing and Duration Dilemmas	Determining effective dosing and assessing long-term impacts is essential, with concerns around toxicity and therapeutic duration.

7	Patient-Specific Variability	Genetic and physiological differences require tailored strategies to ensure consistent therapeutic outcomes across diverse patient populations.
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Future Prospects of siRNA Therapeutics

The future of siRNA lies in expanding beyond liver targets through innovative delivery methods and personalized approaches, positioning it as a key player in next-generation precision medicine⁴⁰.

Conclusion:

siRNA has revolutionized gene silencing, transitioning from a powerful research tool to a clinical therapeutic platform. Despite inherent limitations, continuous innovations in molecular design and delivery systems have substantially enhanced its viability. The success of drugs like Inclisiran and Patisiran underscores the therapeutic promise of siRNA. Future breakthroughs in targeting, safety, and scalability will determine its role in mainstream medicine. As the field evolves, siRNA is poised to reshape the landscape of disease management and personalized therapy.

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