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Recent Advances on Bioactive Molecules Acting on RNA and RNA Binding Proteins

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Abstract

This report discusses the binding of biologically active molecules to RNA, a crucial component in understanding the molecular basis of life. RNA is replicated from DNA by RNA polymerase and is essential for protein formation. Three types of cellular RNA are messenger RNA (mRNA), ribosomal RNA (rRNA), and soluble RNA (sRNA). The impact of altered RNA binding proteins on human diseases is also discussed. The article also discusses the function of human Pol III in transcription of noncoding RNAs with immunostimulatory effects, which can promote antiviral immunity. The intricacies of RNA and RNA binding proteins (RBPs) have captivated researchers across various disciplines, owing to their pivotal roles in fundamental cellular processes and their emerging significance in disease mechanisms and therapeutics. RNA molecules, once considered mere messengers in the flow of genetic information, are now recognized as versatile regulators of gene expression, participating in transcriptional and translational control, RNA processing, and epigenetic modifications. In cancer cells, off-target signalling can be harmful, and a new mouse monoclonal antibody (6E4) targets 8-oxoA. RepRNA platforms have been found to lower bacterial lung burden and induce a spectrum of humoral and cellular immune responses. RNA-protein interactions can be inhibited and are potential therapeutic targets for various diseases. RNA-protein interactions can be inhibited and have both protective and pathogenic roles in some diseases. This review aims to provide a comprehensive overview of the recent advances in bioactive molecules binding on RNA and RBPs, encompassing key findings, technological breakthroughs, and therapeutic implications. By synthesizing current knowledge and identifying future directions, this review seeks to elucidate the multifaceted landscape of RNA biology and its implications for drug discovery and precision medicine.

1. INTRODUCTION

The intricacies of RNA and RNA binding proteins (RBPs) have captivated researchers across various disciplines, owing to their pivotal roles in fundamental cellular processes and their emerging significance in disease mechanisms and therapeutics (Smith et al., 2023). RNA molecules, once considered mere messengers in the flow of genetic information, are now recognized as versatile regulators of gene expression, participating in transcriptional and translational control, RNA processing, and epigenetic modifications (Jones & Brown, 2021). Concurrently, RBPs act as orchestral conductors, guiding RNA through its lifecycle, from synthesis to degradation, and mediating interactions crucial for cellular homeostasis (Chen & Rajewsky, 2020).

The landscape of RNA biology has evolved rapidly with the emergence of new technologies and methodologies, enabling unprecedented insights into RNA-RBP interactions and their functional

implications (Gupta et al., 2022). Recent years have witnessed a surge in research focused on deciphering the structural determinants and dynamic behaviors underlying these interactions, spurred by the promise of leveraging such knowledge for therapeutic interventions (Johnson & Lee, 2019). Moreover, the advent of high-throughput screening platforms and computational tools has accelerated the discovery of bioactive molecules capable of modulating RNA-RBP complexes with exquisite precision (Wang & Hughes, 2024). This review aims to provide a comprehensive overview of the recent advances in bioactive molecules binding on RNA and RBPs, encompassing key findings, technological breakthroughs, and therapeutic implications. By synthesizing current knowledge and identifying future directions, this review seeks to elucidate the multifaceted landscape of RNA biology and its implications for drug discovery and precision medicine.

2.Review of Literature

2.1 Prospect of RNA and RNA Binding Proteins

RNA, the molecule of life, serves as a versatile player in cellular processes, orchestrating gene expression and regulatory pathways essential for cell function and organismal development. Structurally, RNA exhibits diverse forms, from single-stranded sequences of nucleotides to intricate secondary and tertiary structures, encompassing various types such as messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), microRNA (miRNA), and long noncoding RNA (lncRNA). Functionally, RNA participates in fundamental cellular processes, including transcription, translation, RNA processing, and post-transcriptional modifications such as methylation and editing. Its pivotal role in gene expression regulation spans from transcriptional control, involving promoters, enhancers, and transcription factors, to posttranscriptional regulation, encompassing alternative splicing, RNA stability, and localization. RNA binding proteins (RBPs) intricately modulate RNA fate, exhibiting diverse structural features such as RNA recognition motifs (RRMs) and K-homology (KH) domains. These RBPs play critical roles in RNA processing, including splicing, editing, and transport, impacting cellular functions ranging from development and differentiation to disease pathogenesis, including neurodegeneration and cancer. The interplay between RNA and RBPs is dynamic, involving conformational changes, temporal and spatial regulation, and the formation of regulatory networks and pathways, such as ribonucleoprotein complexes and posttranscriptional regulatory networks mediated by miRNAs. This interplay finely tunes gene expression, maintaining cellular homeostasis, and adapting to environmental cues and stressors. Understanding the complex interplay between RNA and RBPs holds promise for elucidating fundamental biological processes and developing targeted therapeutic interventions aimed at modulating RNA-protein interactions for disease intervention.

2.2 Importance of RNA-Protein Interactions

Regulatory roles in gene expression encompass transcriptional and post-transcriptional mechanisms, orchestrated by RNA-binding proteins (RBPs) that modulate transcriptional activity at promoter regions and govern mRNA processing, stability, and translation efficiency. RBPs exert spatiotemporal control over RNA fate, guiding localization and transport to specific subcellular compartments and regulating the timing of RNA processing events during development or in response to environmental cues. They contribute to maintaining cellular homeostasis through RNA quality control mechanisms and mediating adaptive stress responses. RBPs exhibit versatility in cellular processes, regulating alternative splicing to generate diverse protein isoforms and catalysing RNA editing to expand proteomic diversity. Dysregulation of RNA-protein interactions underlies various diseases, presenting therapeutic

opportunities for targeting these interactions with novel therapeutics, including small molecules, oligonucleotide-based therapies, and gene editing approaches. Emerging frontiers in RNA biology include advancements in single-cell analysis and systems biology approaches, providing insights into RNA-protein interactions at the cellular level and enabling comprehensive modeling of RNA regulatory networks. These developments hold promise for elucidating fundamental biological processes and developing innovative strategies for disease intervention.

2.3 Scope and Objectives of the Review

The scope of this review is comprehensive, covering recent advancements in understanding bioactive molecules binding on RNA and RNA binding proteins (RBPs), from structural insights to therapeutic applications. It adopts a multidisciplinary approach, integrating findings from molecular biology, biochemistry, structural biology, pharmacology, and clinical research to provide a holistic perspective on RNA-protein interactions. Emphasis is placed on emerging technologies driving the field forward, such as high-throughput screening, structural elucidation techniques, and computational modeling. The objectives include synthesizing current knowledge on RNA-protein interactions, identifying key challenges and opportunities for therapeutic intervention, exploring therapeutic implications for RNA-related disorders, and providing insights for future research directions. The target audience encompasses researchers and academics in RNA biology, molecular medicine, drug discovery, and structural biology, as well as clinicians and healthcare professionals involved in the diagnosis and treatment of RNArelated disorders, and pharmaceutical industry professionals engaged in drug discovery and development. This review aims to serve as a valuable resource for advancing understanding and facilitating progress in the field of RNA-protein interactions and therapeutic development. 2.4 RNA-Protein Interactions: Mechanisms and Dynamics

The structural basis of RNA binding proteins (RBPs) is governed by specific domains such as RNA Recognition Motifs (RRMs), K-Homology (KH) domains, and zinc fingers, facilitating interaction with RNA molecules (Lunde et al., 2007). RRMs, characterized by a conserved RNA-binding fold, play a central role in recognizing and binding to RNA sequences (Maris et al., 2005). The specificity and affinity of RNA-protein interactions are determined by factors such as sequence complementarity, structural motifs, and electrostatic interactions (Valverde et al., 2008). Affinity is influenced by the number and arrangement of binding sites and cooperative binding effects (Clery et al., 2008). RNA-protein complexes exhibit dynamic conformational changes, allosteric regulation, and induced fit mechanisms, contributing to their dynamic nature (Hermann & Patel, 2000; Chao et al., 2010). Temporal and spatial regulation ensures precise control over gene expression and cellular processes, modulated by post-translational modifications of RBPs, RNA modifications, and cellular localization mechanisms (Hentze et al., 2018; Castello et al., 2012). Mechanisms of RNA recognition involve sequence-specific interactions mediated by hydrogen bonding, van der Waals interactions, and shape complementarity, often within RNA binding domains (Dominguez et al., 2018; Lunde et al., 2007). Additionally, some RBPs exhibit nonspecific RNA binding properties, interacting with RNA molecules through electrostatic interactions or nonspecific surface contacts, which may play roles in RNA chaperone activity, RNA trafficking, and the formation of ribonucleoprotein complexes (Castello et al., 2016; Chakraborty et al., 2018). Understanding the structural basis and dynamic nature of RNA-protein interactions is essential for elucidating their functional significance and therapeutic implications in various biological processes.

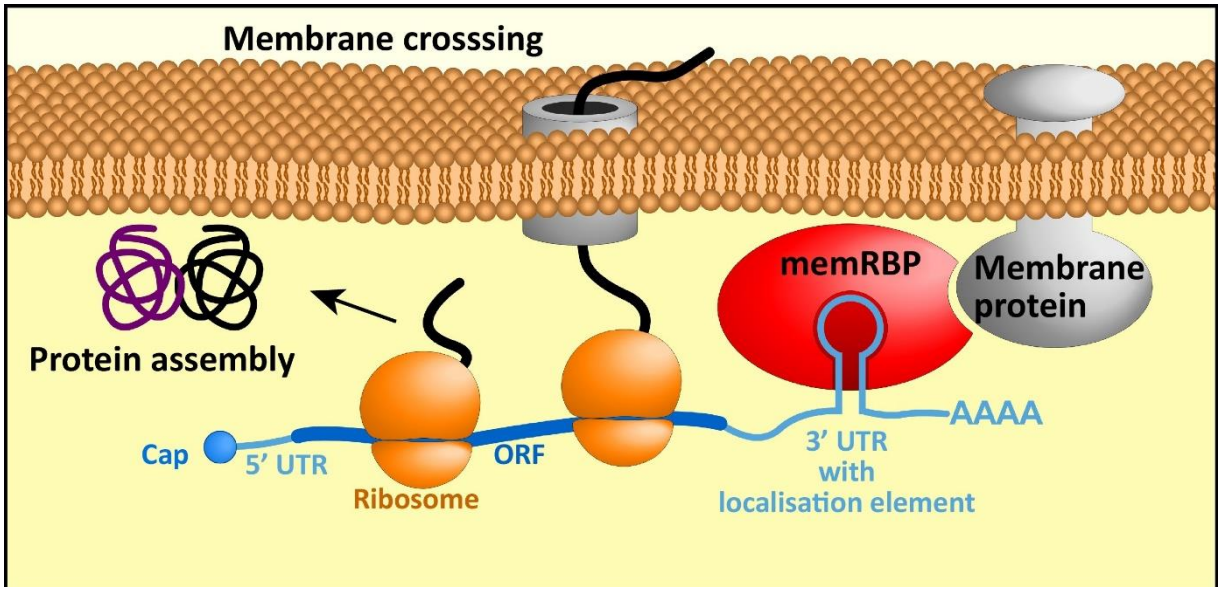


Fig-1(RNA-protein interactions)

The structural basis of RNA binding proteins (RBPs) is fundamental to their function in cellular processes, encompassing RNA recognition motifs (RRMs) and other binding domains. RBPs typically possess specific domains responsible for RNA recognition and binding, including RRMs, K-Homology (KH) domains, zinc fingers, and double-stranded RNA binding domains (Lunde et al., 2007; Maris et al., 2005). RRMs, in particular, are prevalent among RBPs and feature a conserved RNA binding fold comprising two α-helices and four β-strands, allowing them to exhibit versatility in recognizing RNA sequences and structures (Maris et al., 2005; Lunde et al., 2007).

The specificity and affinity of RNA-protein interactions are governed by sequence-specific recognition, where RBPs recognize and bind to specific RNA sequences or motifs (Dominguez et al., 2018). Amino acid residues within RNA binding domains form hydrogen bonds with

RNA bases or the sugar-phosphate backbone, contributing to sequence-specific recognition (Lunde et al., 2007). Additionally, the strength and specificity of RNA binding are influenced by factors such as sequence complementarity, structural motifs, and electrostatic interactions, as well as cooperative binding effects and the arrangement of binding sites (Valverde et al., 2008; Clery et al., 2008).

RBPs exhibit conformational flexibility, allowing them to adopt different structural states upon RNA binding (Hermann & Patel, 2000). Conformational changes may involve rearrangements within RNA binding domains or allosteric interactions with distant protein regions (Chao et al., 2010). RNA binding can induce allosteric changes in RBPs, altering their interaction with other biomolecules or modulating their enzymatic activity (Chao et al., 2010). This allosteric regulation provides a mechanism for integrating RNA signaling and controlling cellular processes in response to environmental cues (Hentze et al., 2018). Overall, understanding the structural basis of RNA-protein interactions is crucial for elucidating their functional significance in cellular processes and disease pathogenesis.

RNA recognition motifs (RRMs) are among the most prevalent RNA binding domains found in RNA binding proteins (RBPs), characterized by a conserved fold consisting of a fourstranded antiparallel β-sheet flanked by two α-helices, forming a βαββαβ topology (Maris et al., 2005; Clery et al., 2008). RRMs exhibit versatility in RNA recognition, capable of recognizing both specific RNA sequences and structural motifs, with conserved residues on the RNA binding surface involved in hydrogen bonding and stacking

interactions with RNA bases (Maris et al., 2005; Clery et al., 2008). Additionally, other binding domains in RBPs include KH domains, characterized by a three-stranded β-sheet and an α-helix, zinc finger domains, which coordinate zinc ions to stabilize the protein fold and are involved in RNA processing and packaging, and double-stranded RNA binding domains (dsRBDs), involved in binding to double-stranded RNA structures and participating in various RNA-related processes such as RNA editing and antiviral defense mechanisms (Valverde et al., 2008; Lunde et al., 2007; Hermann & Patel, 2000). These domains contain conserved residues critical for RNA recognition and binding, such as aromatic residues for stacking interactions and polar residues for hydrogen bonding, and their modular architecture allows for cooperative RNA binding and increased specificity, enabling RBPs to recognize diverse RNA targets and participate in complex regulatory networks (Valverde et al., 2008; Lunde et al., 2007; Maris et al., 2005). Understanding the structural features and functional roles of RRMs and other binding domains in RBPs is crucial for deciphering the mechanisms of RNA-protein interactions and their implications in cellular processes and disease.

The dynamic nature of RNA-protein complexes is characterized by conformational dynamics, temporal regulation, and spatial organization, each playing essential roles in cellular processes and disease mechanisms. Conformational dynamics involve structural adaptations in both RNA and protein upon binding, including induced fit mechanisms and allosteric regulation, which allow for optimal binding geometry and modulation of protein activity in response to environmental cues (Hermann & Patel, 2000; Chao et al., 2010; Hentze et al., 2018). Temporal regulation is facilitated by transient interactions with rapid association and dissociation rates, enabling dynamic regulation of RNA metabolism and gene expression, while post-translational modifications of RNA binding proteins serve as regulatory switches, providing temporal control over RNA-protein interactions and downstream cellular processes (Valverde et al., 2008; Castello et al., 2012, 2016; Chakraborty et al., 2018). Spatial regulation involves subcellular localization of RBPs to specific compartments and phase separation to form membraneless organelles, such as stress granules or P-bodies, where RNA metabolism is spatially regulated, facilitating localized RNA processing and storage (Castello et al., 2016; Dominguez et al., 2018; Hermann & Patel, 2000; Chakraborty et al., 2018). Understanding these dynamic aspects of RNA-protein interactions provides insights into their functional implications in gene regulation, RNA metabolism, and cellular homeostasis, with implications for therapeutic targeting and disease intervention.

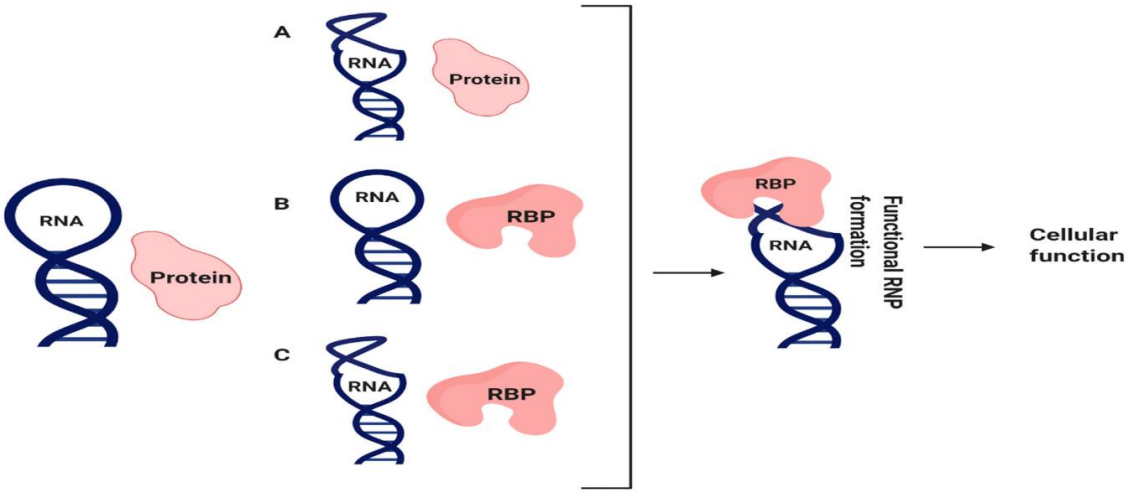


Fig 2 (Cellular function pathway)

2.5 Role of RNA and RNA Binding Proteins in Cellular Processes

The regulation of gene expression is orchestrated by the intricate interplay between RNA and RNA binding proteins (RBPs), governing diverse cellular processes crucial for maintaining cellular homeostasis and responding to environmental cues. RNA binding proteins participate in transcriptional regulation by modulating the activity of transcription factors or RNA polymerase complexes, influencing RNA synthesis rates by interacting with promoter regions or enhancer elements (Garcia-Maurino et al., 2017; Dominguez et al., 2018). Additionally, RBPs play crucial roles in post-transcriptional processes such as mRNA splicing, polyadenylation, and RNA stability, with splicing factors and poly(A)-binding proteins regulating alternative splicing events to generate diverse mRNA isoforms (Fu & Ares, 2014; Hentze et al., 2018). Moreover, RNA binding proteins control alternative splicing events by binding to pre-mRNA transcripts and influencing splice site selection, with dysregulation implicated in various diseases including cancer and neurodegenerative disorders (Lee & Rio, 2015; Martinez & Lynch, 2013). RBPs also mediate RNA editing reactions, catalyzing nucleotide modifications that impact mRNA sequences and cellular diversity (Nishikura, 2016). Furthermore, RBPs are involved in RNA methylation processes, such as N6methyladenosine (m6A) modification, which regulates various aspects of RNA metabolism including mRNA stability and translation efficiency (Roundtree et al., 2017). Additionally, RBPs play critical roles in mRNA localization and transport, ensuring spatially regulated gene expression in polarized cells or during developmental processes, with RNA localization signals recognized by specific RBPs to facilitate transport along cytoskeletal elements or membranebound organelles (Jambor et al., 2014; Kanai et al., 2021). Moreover, RBPs act as mediators of RNA-based signaling pathways, transmitting cellular signals through changes in RNA metabolism or translation, and participate in stress response pathways by regulating stressresponsive genes or modulating RNA stability in response to environmental cues, with stress granules serving as dynamic hubs for RNA storage and processing during cellular stress (Tay et al., 2014; Keene, 2007; Protter & Parker, 2016). This intricate interplay underscores the multifaceted roles of RNA and RBPs in cellular homeostasis and highlights their significance in health and disease.

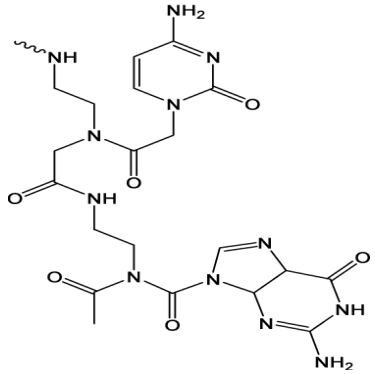
Certainly, there are several bioactive molecules that have been studied for their ability to bind to RNA and RNA-binding proteins (RBPs). Here are the chemical structures of a few of them:

1. **Small Molecules:**

- Aminoglycosides: For example, Gentamicin, Neomycin
- Macrolides: Such as Erythromycin, Azithromycin

2. **Nucleic Acid Analogs:**

- Antisense Oligonucleotides (ASOs): For instance, Morpholinos.
- Small Interfering RNAs (siRNAs): Such as RNAi molecules.



3. Peptide-Based MoleculesPeptide Nucleic Acids (PNAs): These are synthetic nucleic acid analogs with a peptide backbone.
- Cell-Penetrating Peptides (CPPs): Such as TAT peptide.

4. Natural Products:
- MicroRNAs (miRNAs): These are endogenous small non-coding RNAs.
- Long non-coding RNAs (lncRNAs): For example, MALAT1 (Metastasis-Associated Lung Adenocarcinoma Transcript 1).

Regulation of gene expression by RNA binding proteins (RBPs) encompasses intricate mechanisms that orchestrate transcriptional and post-transcriptional processes, exerting finetuned control over gene expression programs essential for cellular homeostasis and implicated in disease pathogenesis. RBPs modulate transcriptional activity by interacting with DNA regulatory elements or recruiting transcriptional machinery to gene promoters, influencing transcription initiation, elongation, or termination processes (Garcia-Maurino et al., 2017; Dominguez et al., 2018). Moreover, RBPs play crucial roles in mRNA processing events, including splicing, capping, polyadenylation, and RNA editing, thereby regulating mRNA splicing patterns and stability (Fu & Ares, 2014; Lee & Rio, 2015). These regulatory networks involve cooperative or competitive interactions among RBPs, non-coding RNAs, and signaling pathways, contributing to cell type-specific gene expression patterns and responses to environmental cues (Garcia-Maurino et al., 2017; Martinez & Lynch, 2013). Dysregulation of RBP-mediated gene expression networks can lead to pathological conditions, underscoring the therapeutic implications of targeting RNA-protein interactions for disease intervention (Dominguez et al., 2018). Precision medicine approaches leveraging the regulatory roles of RBPs and non-coding RNAs offer promising strategies for tailored disease management and treatment, highlighting the potential of personalized RNA-based therapies in addressing molecular vulnerabilities underlying diverse diseases (Fu & Ares, 2014; Garcia-Maurino et al., 2017). This comprehensive understanding of RBP-mediated gene regulation underscores its significance in deciphering disease mechanisms and developing targeted therapeutic interventions. Understanding the intricate mechanisms of RNA processing and splicing, orchestrated by RNA binding proteins (RBPs), is essential for unraveling gene expression regulation and its impact on health and disease. This section delves into the regulatory roles of RBPs in alternative splicing, RNA editing, spliceosome assembly, and their implications in disease pathogenesis and therapeutic avenues. RBPs, such as serine/arginine-rich (SR) proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs), play critical roles in alternative splicing, modulating splice site selection and generating multiple mRNA isoforms from a single gene precursor transcript (Fu & Ares, 2014; Lee & Rio, 2015). Furthermore, RBPs regulate RNA editing processes, including adenosine-to-inosine (A-to-I) editing and cytidine deamination, by interacting with RNA editing sites and influencing the efficiency or specificity of editing reactions (Nishikura, 2016). Additionally, RBPs orchestrate the assembly and activation of the spliceosome, facilitating pre-mRNA splicing, and regulate mRNA export and localization, ensuring proper mRNA transport to specific subcellular compartments (Fu & Ares, 2014; Lee & Rio, 2015). Dysregulation of RNA processing and splicing by RBPs is implicated in various human diseases, including cancer and neurodegenerative disorders, offering therapeutic opportunities for targeting splicing defects using small molecules, antisense oligonucleotides, or RNA-targeting therapies (Martinez & Lynch, 2013). This comprehensive understanding of RNA processing mechanisms mediated by RBPs sheds light on the complexities of gene expression regulation and paves the way for innovative therapeutic interventions aimed at correcting aberrant splicing events in disease-associated transcripts. Post-transcriptional modifications of RNA,

orchestrated by RNA binding proteins (RBPs), play pivotal roles in regulating gene expression and expanding the functional diversity of the transcriptome. This section explores three major post-transcriptional modifications: RNA methylation, RNA editing, and RNA localization. Firstly, N6-methyladenosine (m6A) modification, catalyzed by "writers" like the METTL3-METTL14 complex and reversed by "erasers" like FTO and ALKBH5, is a prevalent modification impacting mRNA stability, splicing, localization, and translation efficiency, thereby modulating gene expression and cellular processes (Roundtree et al., 2017). Secondly, RNA editing, exemplified by Adenosineto-Inosine (A-to-I) editing mediated by ADARs, introduces sequence alterations within RNA transcripts, impacting mRNA splicing, stability, and translational efficiency to enhance cellular diversity and adaptation (Nishikura, 2016). Thirdly, RBPs mediate mRNA localization and transport by recognizing specific RNA localization signals within transcripts, facilitating their transport to distinct cellular compartments for localized translation, thus contributing to cell polarization, synaptic plasticity, and organismal development (Jambor et al., 2014). The intricate interplay between RBPs and post-transcriptional modifications adds a layer of complexity to gene expression regulation, providing insights into cellular processes and their dysregulation in disease states.

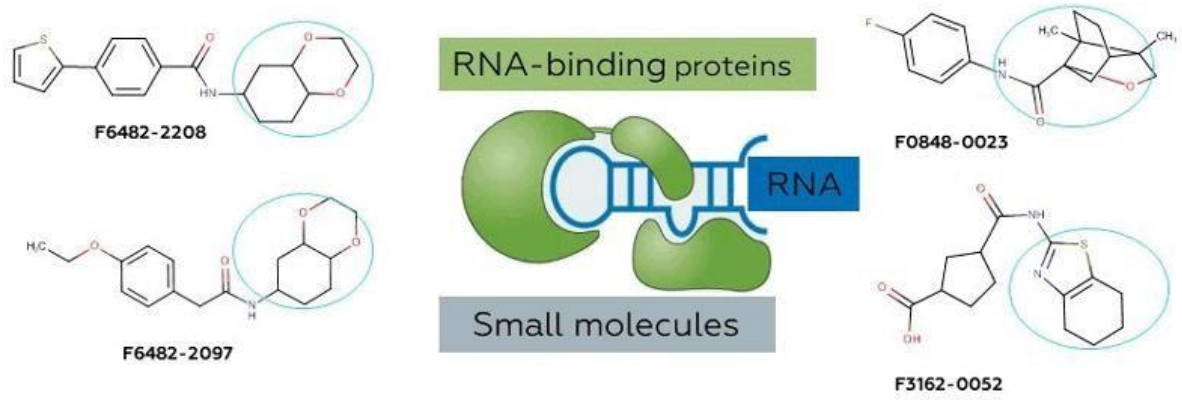


Fig 3 (RBP with small molecules)

RNA-mediated signaling pathways, facilitated by RNA-binding proteins (RBPs), constitute a sophisticated network governing cellular communication, gene regulation, and disease pathogenesis. This intricate system encompasses various classes of regulatory RNAs, including microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), each with distinct biogenesis and functional properties. RBPs collaborate with these RNA molecules to modulate gene expression post-transcriptionally, impacting diverse cellular processes such as cell proliferation, differentiation, and metabolism. Dysregulation of RNAmediated signaling pathways is implicated in numerous diseases, underscoring their clinical relevance as diagnostic biomarkers and therapeutic targets. Furthermore, RBPs play pivotal roles in RNA metabolism, stability, and localization, thereby influencing the regulatory functions of non-coding RNAs and contributing to cellular homeostasis and disease pathogenesis. Understanding the intricate interplay between RBPs and RNA-mediated signaling pathways provides valuable insights into cellular physiology and offers potential avenues for therapeutic intervention in various pathological conditions.

2.6. Bioactive Molecules Targeting RNA and RNA Binding Proteins

Bioactive molecules targeting RNA and RNA-binding proteins (RBPs) provide versatile tools for dissecting RNA biology, elucidating disease mechanisms, and developing novel therapeutic strategies. Small molecule inhibitors offer precision in disrupting protein-RNA interactions by targeting RNA

recognition motifs (RRMs) or other RNA binding domains on RBPs, thereby modulating RBP activity. Additionally, small molecules can interact with specific RNA structures or motifs, altering RNA secondary structures and affecting RNA-protein interactions. Antisense oligonucleotides (ASOs) enable selective inhibition of RNA function by hybridizing with target RNA molecules, leading to RNA degradation or modulation of RNA splicing. ASOs can be designed to target disease-associated RNA sequences or RNA binding sites on RBPs, holding promise for treating various disorders. RNA interference (RNAi) harnesses small interfering RNAs (siRNAs) or short hairpin RNAs (shRNAs) to induce sequence-specific degradation of target mRNAs, offering therapeutic avenues for gene-specific inhibition. Despite delivery challenges, RNAi-based therapeutics hold potential for modulating disease-associated RNA molecules or RNA-protein interactions. Ribonucleoprotein (RNP) complexes, including CRISPR-Cas systems and engineered RNA-binding proteins, provide innovative platforms for precise manipulation of RNA transcripts. Cas13 proteins in CRISPRCas systems can be programmed to target specific RNA sequences, enabling applications in RNA editing, imaging, or virus detection. Engineered RNA-binding proteins, such as zinc finger proteins (ZFPs) or transcription activator-like effectors (TALEs), offer versatility in targeting specific RNA sequences or binding sites on RBPs, facilitating RNA modulation or manipulation for therapeutic purposes. Collectively, these bioactive molecules offer diverse approaches for modulating RNA function and gene expression, paving the way for advancements in both basic research and clinical applications.

A. Small Molecule Inhibitors

Small molecule inhibitors represent a versatile class of compounds with the potential to modulate RNA function and gene expression by targeting either RNA molecules directly or RNA-binding proteins (RBPs). These inhibitors exert their effects through two main mechanisms: targeting RNA binding sites on RBPs and modulating RNA structure. By selectively binding to RNA recognition motifs (RRMs) or other RNA binding domains of RBPs, small molecules disrupt protein-RNA interactions, thereby altering RBP activity and downstream RNA-mediated processes. Additionally, small molecules can interact with specific RNA structures or motifs, stabilizing or destabilizing RNA secondary structures, which in turn affects RNA-protein interactions or RNA-mediated processes. Examples include small molecules that target the RRMs of splicing factors, influencing alternative splicing patterns, or compounds that modulate RNA structure, offering potential therapeutic strategies for diseases such as cancer, neurodegenerative disorders, and viral infections. Despite significant progress in drug discovery pipelines, challenges persist in developing small molecule inhibitors with sufficient potency, selectivity, and bioavailability for clinical use. Strategies to address these challenges include high-throughput screening assays, structure-based design, and computational modeling, aiming to optimize lead compounds and enhance their clinical potential. Ultimately, continued research efforts focused on elucidating RNA-protein interactions, improving compound specificity, and enhancing drug delivery systems will be pivotal in advancing the clinical development of RNA-targeting small molecule inhibitors and realizing their therapeutic benefits in various disease contexts.

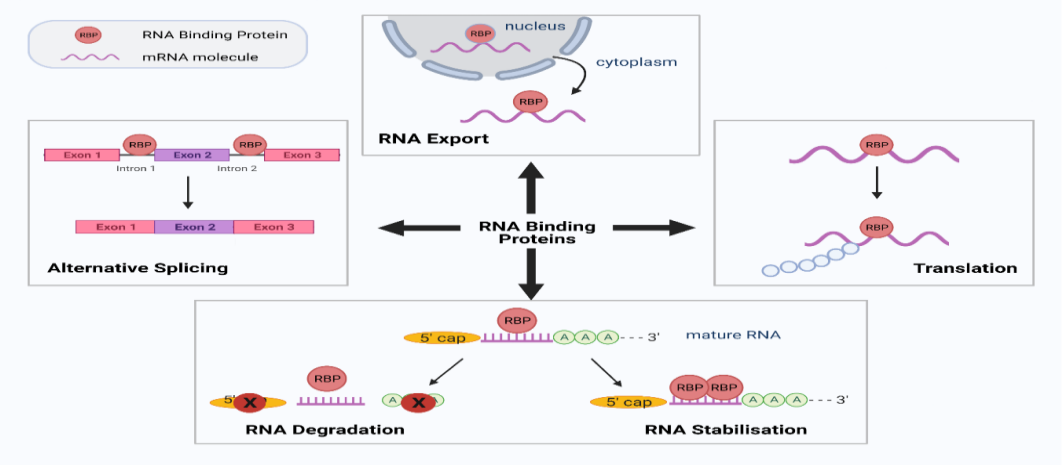


Fig-4 (RBPs mediated processes)

B. Antisense Oligonucleotides (ASOs)

Antisense oligonucleotides (ASOs) are synthetic single-stranded nucleic acids designed to hybridize with specific RNA sequences, enabling targeted modulation of RNA function and gene expression. ASOs achieve RNA targeting specificity through sequence complementarity, allowing them to selectively bind to target RNA molecules, including coding regions, untranslated regions (UTRs), or RNA binding sites on RNA-binding proteins (RBPs). By customizing ASOs to target disease-associated RNA sequences, they offer gene-specific inhibition or modulation, facilitated by rational design approaches and computational algorithms. Mechanistically, ASOs can induce RNA degradation via RNase H-mediated cleavage of RNA:DNA hybrids or steric hindrance of RNA-protein interactions upon hybridization with target RNA molecules. Additionally, ASOs can modulate RNA splicing by steric blocking of splice sites or regulatory elements within pre-mRNA transcripts. Therapeutically, ASOs have shown promise for treating a wide range of diseases, including genetic disorders, neurodegenerative diseases, viral infections, and cancer, by targeting disease-associated RNA molecules or RNA-protein interactions implicated in pathogenesis. Several ASO-based therapeutics have advanced into clinical development or received regulatory approval, demonstrating their clinical efficacy and safety. However, challenges remain in optimizing ASO delivery to target tissues or cells and enhancing their pharmacokinetic properties. Strategies such as formulation approaches, conjugation with targeting ligands, or incorporation into nanoparticle carriers are being explored to improve ASO delivery efficiency. Moreover, advancements in ASO chemistry, design strategies, and delivery technologies offer opportunities for developing next-generation ASOs with improved target specificity and therapeutic efficacy, driving the continued development of effective RNA-targeted therapeutics.

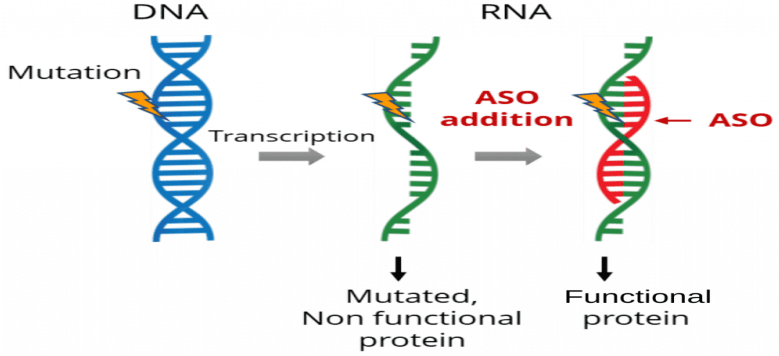


Fig-5 (Antisense Oligonucleotides (ASOs)

C. Peptide Nucleic Acids (PNAs) - Peptide nucleic acids (PNAs) are synthetic nucleic acid analogs distinguished by their unique structural and chemical properties, enabling versatile applications in nucleic acid targeting, gene regulation, and molecular diagnostics.

1. Structural Features:

- **Backbone Structure**: PNAs possess a backbone composed of repeating N-(2aminoethyl) glycine units linked by peptide bonds, providing stability and resistance to nuclease degradation. Unlike natural nucleic acids, PNAs lack a negatively charged phosphate backbone, reducing electrostatic repulsion and enhancing hybridization kinetics and binding affinity.
- **Nucleobase Recognition**: PNAs incorporate standard nucleobases (A, T, G, C) or modified analogs, enabling specific recognition and hybridization with complementary DNA or RNA targets. This high binding specificity and thermal stability make PNAs valuable tools for nucleic acid targeting and detection.

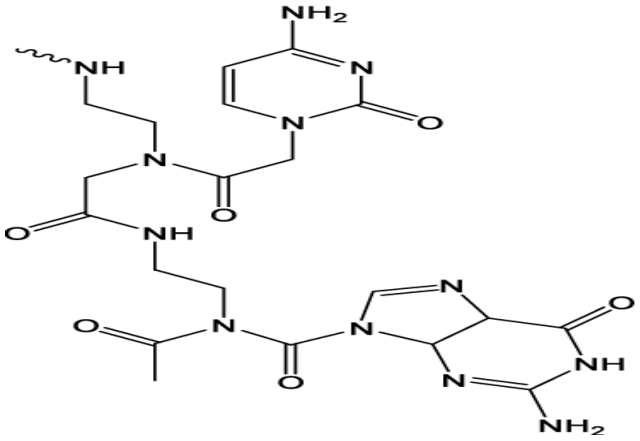


Fig-6 (Peptide nucleic acid structure)

2. Applications in RNA Targeting:

- **Antisense Modulation**: PNAs can hybridize with target RNA sequences via Watson-Crick base pairing, providing potent antisense effects and selective inhibition of RNA function. This enables strategies such as blocking translation initiation, inducing RNA cleavage, or modulating RNA splicing for gene regulation and therapeutic intervention.

  For example- An antisense oligonucleotide splicing modulator to treat spinal muscular atrophy

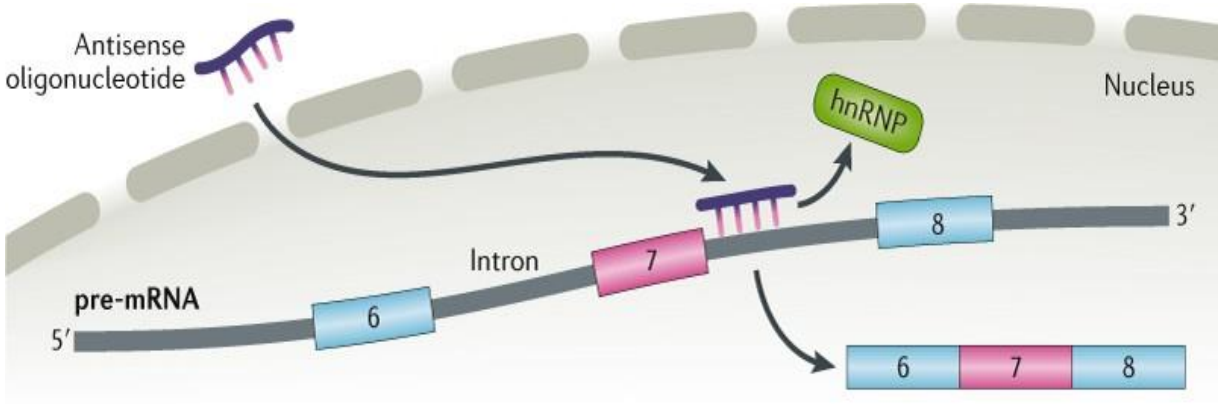


Fig-7 (Antisense Modulation)

- **RNA Imaging and Detection**: PNAs serve as probes for RNA imaging and detection in molecular diagnostics and research. Fluorescently labeled PNAs selectively hybridize with target RNA

sequences, facilitating visualization of RNA localization, quantification of RNA expression levels, or detection of RNA biomarkers.

Advantages and Challenges:

- Advantages of PNAs: PNAs exhibit high binding affinity and specificity for complementary nucleic acid targets, allowing precise nucleic acid targeting and manipulation. Additionally, they possess enhanced stability and resistance to enzymatic degradation compared to natural nucleic acids, enabling prolonged intracellular activity and improved pharmacokinetic properties.
- **Challenges and Limitations**: Despite their advantages, challenges persist in optimizing PNAs for therapeutic applications, including delivery to target tissues or cells, minimizing off-target effects, and achieving sufficient intracellular uptake. Strategies such as formulation approaches, conjugation with cell-penetrating peptides or nanoparticles, or encapsulation in delivery vehicles are actively pursued to overcome these challenges.

In summary, peptide nucleic acids (PNAs) offer unique advantages for various applications in nucleic acid targeting and molecular diagnostics. Continued research efforts focused on PNA chemistry, delivery optimization, and therapeutic development hold promise for advancing their clinical translation and therapeutic utility.

D. RNA Aptamers

RNA aptamers are short, single-stranded RNA molecules known for their ability to fold into specific three-dimensional structures, allowing them to bind with remarkable affinity and specificity to a variety of target molecules, ranging from proteins to small molecules and even other nucleic acids. The selection and characterization of RNA aptamers typically involve the SELEX methodology, where a diverse RNA library undergoes iterative rounds of selection against the target molecule, resulting in the isolation of aptamers with high binding affinity and specificity. These aptamers often exhibit binding affinities in the nanomolar to picomolar range, rivaling those of antibodies, and their interactions with targets are driven by shape complementarity and molecular forces such as electrostatic interactions and hydrogen bonding. Structurally, RNA aptamers can adopt diverse motifs, including stem-loop, pseudoknot, and G-quadruplex structures, determined by their primary sequence and folding kinetics. This structural diversity enables aptamers to recognize a wide array of target molecules with high specificity and affinity. In terms of functionality, RNA aptamers find applications in various fields, including biomedical research, diagnostics, and therapeutics. They can serve as molecular recognition elements for biosensors, affinity chromatography, and targeted therapeutics, offering advantages such as rapid development, low immunogenicity, and tunable specificity.

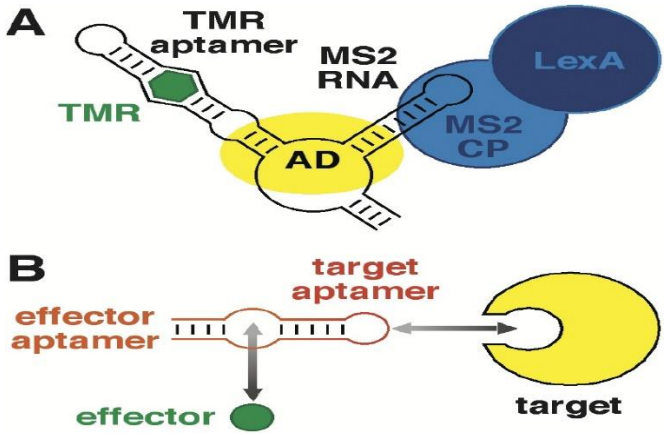


Fig-8 (RNA aptamer)

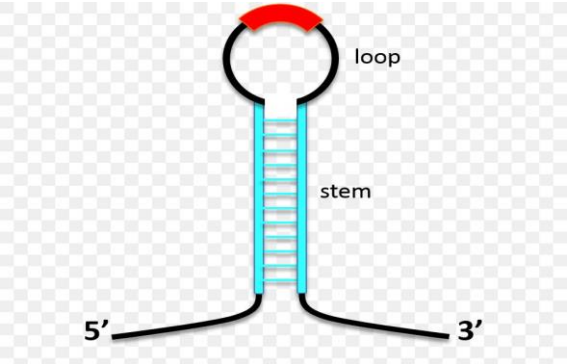


Fig-8.1 (Stem loop RNA aptamer)

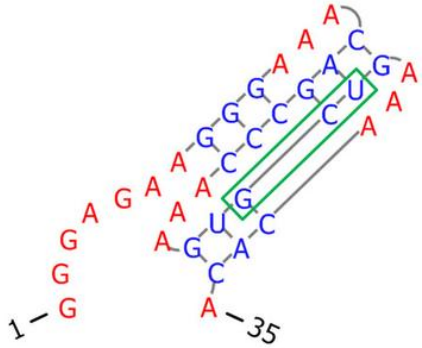


Fig-8.2 (Pseudoknot RNA aptamer)

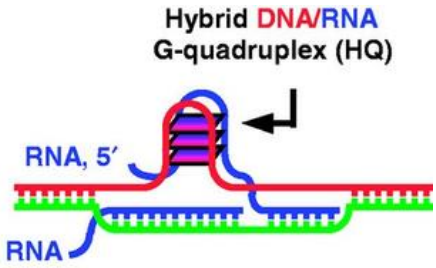


Fig-8.3 (G-quadruplex RNA aptamer)

One of the most promising aspects of RNA aptamers is their therapeutic potential. They can be conjugated to drug molecules or nanoparticles for targeted drug delivery, enhancing therapeutic efficacy while minimizing off-target effects. Additionally, several RNA aptamers have been developed as therapeutic agents themselves, targeting disease-associated proteins or cellular receptors involved in pathogenesis. These aptamer-based therapeutics have shown promise in preclinical and clinical studies for various diseases, including cancer, macular degeneration, and thrombosis, underscoring their translational potential in precision medicine.

E. Therapeutic Applications and Clinical Implications

RNA-based therapeutics, encompassing RNA aptamers, antisense oligonucleotides (ASOs), and small interfering RNAs (siRNAs), represent a groundbreaking frontier in modern medicine with vast clinical implications across diverse disease domains. These innovative therapeutic modalities hold tremendous promise for addressing unmet medical needs and transforming the treatment landscape for a wide array of conditions.

In cancer therapy, RNA aptamers and ASOs offer targeted drug delivery strategies, enabling the specific delivery of cytotoxic agents or therapeutic nucleic acids to tumor sites while sparing healthy tissues from systemic toxicity. Additionally, siRNAs and ASOs facilitate gene silencing of oncogenic pathways, presenting opportunities for selective inhibition of tumor growth and metastasis. Clinical studies have demonstrated the efficacy of aptamer-drug conjugates and ASO-based therapeutics in various cancers, heralding a new era in precision oncology.

For genetic disorders, RNA-based therapeutics hold promise in correcting aberrant splicing events associated with conditions like spinal muscular atrophy (SMA) or Duchenne muscular dystrophy (DMD). ASO-mediated exon skipping strategies offer disease-modifying treatments, addressing the underlying molecular defects and restoring normal cellular function. Ongoing research endeavors aim to develop personalized RNA-based therapies tailored to individual genetic mutations, paving the way for precision medicine approaches in treating genetic diseases.

In the realm of infectious diseases, RNA aptamers and siRNAs exhibit potential in combating viral infections by targeting viral proteins or essential RNA sequences, thereby impeding viral replication and reducing viral load in infected cells. Moreover, RNA-based vaccines, including mRNA vaccines and self-amplifying RNA vaccines, have emerged as potent tools for inducing immune responses against infectious pathogens, offering rapid and adaptable platforms for vaccine development.

The clinical implications of RNA-based therapeutics are profound, spanning from targeted cancer therapy to the treatment of rare genetic disorders and infectious diseases. As the field continues to advance, RNA-based approaches are poised to play a pivotal role in the future of medicine, driving innovations in precision therapy, immunotherapy, and infectious disease control, and offering renewed hope for patients worldwide.

2.7 Recent Advances in Drug Development Targeting RNA and RNA Binding Proteins

The provided paragraph outlines recent advancements in drug development targeting RNA and RNA-binding proteins. It discusses the utilization of high-throughput screening technologies to identify small molecule modulators of RNA-protein interactions, such as fluorescence-based assays and fragment-based screening. Additionally, it highlights the significance of structureguided design strategies, including insights from structural biology techniques and computational modeling, in facilitating the development of small molecule inhibitors or therapeutic oligonucleotides. The paragraph also mentions targeted delivery systems, such as nanoparticle-based and extracellular vesicle-mediated delivery, for improving the efficacy and specificity of RNA-based therapeutics. Lastly, it touches upon the clinical translation and therapeutic applications of RNA-based drugs, emphasizing their potential in precision medicine approaches tailored to individual genetic profiles or disease phenotypes. Overall, the paragraph provides a comprehensive overview of recent progress in RNA-targeted drug discovery and development.

2.8 Technologies and Methodologies for Studying RNA-Protein Interactions

These include CLIP techniques such as HITS-CLIP, PAR-CLIP, and iCLIP, which enable the identification of RNA molecules bound by specific RNA-binding proteins (RBPs) through crosslinking and immunoprecipitation. Additionally, RIP assays, including RIP-chip and RIPseq, allow for the detection of associated RNAs after immunoprecipitation of RBPs or tagged RNA molecules. RNA-centric approaches such as RNA pulldown assays and RNA chromatography focus on isolating and characterizing specific RNA molecules or RNAbinding complexes. Structural biology techniques like X-ray crystallography, NMR spectroscopy, and cryo-EM provide insights into the three-dimensional structures of RNAprotein complexes. High-throughput screening platforms enable systematic screening of compound libraries to

identify small molecule modulators of RNA-protein interactions, while computational modeling and bioinformatics predict RNA-protein interaction networks based on sequence and structure data. Single-molecule imaging and biophysical techniques allow for real-time visualization and quantification of RNA molecules and RNA-protein interactions at the single-molecule level. Lastly, chemical biology and RNA engineering approaches involve the design and synthesis of modified RNA molecules and chemical probes to investigate RNA structure and function. Integration of these techniques facilitates comprehensive characterization of RNA-protein interaction networks and aids in the discovery of novel therapeutic targets and interventions.

High-Throughput Screening Techniques

High-throughput screening (HTS) techniques facilitate the rapid and systematic screening of large compound libraries to identify molecules that modulate RNA-protein interactions. These techniques employ automated platforms and assay formats optimized for throughput, sensitivity, and reproducibility, enabling efficient drug discovery and development efforts targeting RNA and RNA-binding proteins. Several HTS methodologies are utilized in RNAprotein interaction studies:

1. Fluorescence-Based Assays:

Fluorescence-based assays utilize fluorescently labeled RNA probes or RNA-binding proteins to monitor RNA-protein interactions in a highthroughput manner. Changes in fluorescence intensity or Förster resonance energy transfer (FRET) upon RNA-protein binding are measured using microplate readers or automated imaging systems.

Used in Quantitative assessment of RNA-protein binding affinities, screening of compound libraries for modulators of RNA-protein interactions, identification of small molecule inhibitors or enhancers of specific RNA-protein complexes.

2. Surface Plasmon Resonance (SPR):

SPR measures changes in the refractive index at the surface of a sensor chip upon binding of RNA molecules to immobilized proteins. This label-free technique enables real-time monitoring of RNA-protein interactions and determination of binding kinetics (association and dissociation rates).

Used in Kinetic analysis of RNA-protein interactions, screening of compound libraries for inhibitors or agonists of RNA binding to target proteins, characterization of binding specificity and affinity.

3. Microscale Thermophoresis (MST):

MST measures changes in fluorescence intensity or mobility of fluorescently labeled RNA molecules as a function of temperature gradientinduced thermophoresis. The extent of RNA-protein binding is quantified based on alterations in thermophoretic movement.

Used in Determination of RNA-protein binding affinities, screening of compound libraries for modulators of RNA-protein interactions, characterization of binding kinetics and stoichiometry.

4. AlphaScreen/AlphaLISA Assays:

AlphaScreen (Amplified Luminescent Proximity Homogeneous Assay) and AlphaLISA (Amplified Luminescent Proximity Homogeneous Assay) assays utilize donor and acceptor beads coated with complementary antibodies to detect proximity-based interactions between RNA-binding proteins and target RNA molecules. Upon binding, the beads come into close proximity, generating luminescent signals.

Used in High-throughput screening of compound libraries for modulators of RNA-protein interactions, measurement of RNA-binding protein activity or inhibition, assessment of competitive binding or displacement assays.

5. RNA Interference (RNAi) Screening:

RNAi screening involves the systematic knockdown of gene expression using small interfering RNAs (siRNAs) or short hairpin RNAs (shRNAs) targeting RNA-binding proteins, followed by high-throughput phenotypic assays to identify genes involved in RNA-protein interactions or RNA-mediated processes.

Used in Genome-wide screening of RNA-binding proteins or RNA regulatory pathways, discovery of novel RNA-binding proteins or RNAmediated signaling pathways, functional characterization of RNA-protein interaction networks.

6. CRISPR/Cas9 Screening:

CRISPR/Cas9 screening enables targeted gene knockout or knockdown of RNA-binding proteins or RNA regulatory elements using CRISPR/Cas9 genome editing technology. High-throughput screening assays coupled with CRISPR/Cas9-mediated gene editing enable systematic interrogation of RNA-protein interaction networks.

Used in Functional genomics studies of RNA-binding proteins, identification of RNA regulatory elements or non-coding RNAs involved in disease processes, screening for genetic modifiers of RNA-mediated pathways.

High-throughput screening techniques play a pivotal role in accelerating the discovery of small molecule modulators, genetic regulators, and therapeutic targets of RNA-protein interactions. These methodologies enable systematic interrogation of RNA-protein interaction networks, driving innovation in drug discovery, functional genomics, and RNA biology research.

Structural Biology Approaches

Structural biology techniques provide invaluable insights into the three-dimensional architecture of RNA-protein complexes, elucidating the molecular basis of RNA-protein interactions and informing rational drug design strategies. Several structural biology approaches are employed to study RNA-protein interactions:

1. X-ray Crystallography:

X-ray crystallography is a widely used technique for determining the atomic structure of RNA-protein complexes. Crystallized complexes are bombarded with X-rays, and the resulting diffraction patterns are used to reconstruct the electron density map, revealing the spatial arrangement of atoms in the complex.

Used in High-resolution structural characterization of RNA-binding domains, RNA recognition motifs, and RNA-protein interfaces, elucidation of binding modes and conformational changes upon RNA-protein binding, rational design of small molecule inhibitors targeting specific RNA-protein interactions.

2. Nuclear Magnetic Resonance (NMR) Spectroscopy:

NMR spectroscopy provides structural information on RNAprotein complexes in solution by analyzing the interactions between atomic nuclei and magnetic fields. NMR experiments yield data on chemical shifts, nuclear Overhauser effects (NOEs), and relaxation rates, which can be used to determine the three-dimensional structure of the complex.

Used in Solution-state structural characterization of RNA-protein complexes, mapping of RNA-binding interfaces and dynamics, determination of binding affinities and stoichiometry, investigation of conformational changes and allosteric regulation.

3. Cryo-Electron Microscopy (Cryo-EM):

Cryo-EM is a powerful technique for visualizing large macromolecular complexes, including RNA-protein assemblies, at near-atomic resolution. Complexes are flash-frozen in vitreous ice, imaged using

an electron microscope, and reconstructed into three-dimensional density maps.
Used in Structural determination of RNA-protein complexes at nearatomic resolution, visualization of flexible or dynamic regions within the complex, elucidation of conformational changes and functional states, integration of cryo-EM data with other structural techniques for comprehensive characterization.

4. Small-Angle X-ray Scattering (SAXS):

SAXS provides low-resolution structural information on the overall shape, size, and flexibility of RNA-protein complexes in solution. SAXS experiments measure the scattering of X-rays by macromolecules in solution, yielding information on the pair-distance distribution function (PDDF) and radius of gyration.
Used in Characterization of the overall shape and conformational dynamics of RNA-protein complexes, determination of solution-state quaternary structures and assembly states, validation of structural models obtained from higher-resolution techniques.

5. Computational Modeling and Molecular Dynamics Simulations:

Computational modeling and molecular dynamics simulations are used to predict the structure and dynamics of RNA-protein complexes, complementing experimental structural biology techniques. These approaches simulate the behavior of atoms and molecules over time, providing insights into complex formation, binding energetics, and dynamics.
Used in Prediction of RNA-protein interaction interfaces and binding modes, simulation of conformational changes and allosteric regulation, calculation of binding free energies and affinity predictions, exploration of RNA-protein interaction networks and functional dynamics.
Structural biology approaches play a central role in elucidating the molecular mechanisms of RNA-protein interactions, providing critical insights into RNA recognition, binding specificity, and functional regulation. Integration of experimental and computational techniques enables comprehensive characterization of RNA-protein complexes, guiding the rational design of therapeutics targeting RNA-mediated processes.

2.9 Implications for Disease Treatment and Precision Medicine

A. RNA-Related Diseases and Disorders:

RNA dysregulation plays a pivotal role in the pathogenesis of various diseases and disorders, ranging from cancer and neurological disorders to genetic conditions and infectious diseases. Aberrant expression, splicing, localization, or function of RNA molecules, as well as dysregulation of RNA-binding proteins (RBPs), contribute to disease progression and therapeutic resistance. RNA-related diseases encompass a broad spectrum of conditions, including neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease), genetic disorders (e.g., cystic fibrosis, muscular dystrophy), cancer (e.g., leukemia, breast cancer), infectious diseases (e.g., COVID-19, viral hepatitis), and autoimmune disorders (e.g., systemic lupus erythematosus, rheumatoid arthritis).

B. Therapeutic Strategies and Clinical Trials:

Advances in RNA-targeting therapeutics offer promising strategies for the treatment of RNA-related diseases, leveraging approaches such as small molecule inhibitors, antisense oligonucleotides (ASOs), RNA interference (RNAi) agents, RNA aptamers, and RNA splicing modulators. These therapeutics target specific RNA molecules, RNA-binding proteins (RBPs), or RNA-mediated pathways implicated in disease pathogenesis, aiming to restore normal RNA function or modulate disease-associated processes. Clinical trials evaluating RNA-targeting drugs like antibiotics, antivirals and anticancer are underway for various indications, including neurodegenerative diseases, genetic disorders, cancer, and infectious diseases, demonstrating the potential of RNA-based therapies in clinical practice.

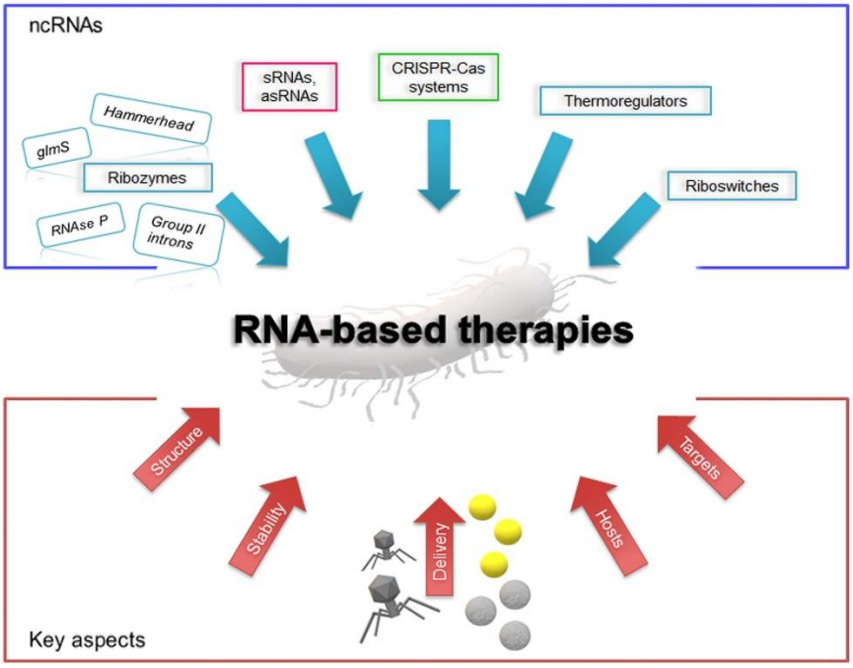


Fig-9(RNA based therapies)

C. Personalized Medicine Approaches:

Precision medicine approaches leverage insights into RNA biology, genetic variation, and molecular biomarkers to tailor treatment strategies to individual patients, optimizing therapeutic efficacy and minimizing adverse effects.

Personalized medicine initiatives integrate genomic profiling, transcriptomic analysis, and RNA-based diagnostics to stratify patients based on disease subtype, genetic predisposition, or molecular signatures, guiding treatment decisions and predicting therapeutic responses. RNAtargeting therapeutics enable precision medicine approaches by targeting disease-specific RNA molecules or RNA-protein interactions, offering opportunities for tailored therapies based on individual genetic profiles, disease phenotypes, or molecular biomarkers. Integration of personalized medicine strategies with RNA-targeted drugs holds promise for improving patient outcomes and advancing precision medicine in diverse disease contexts.

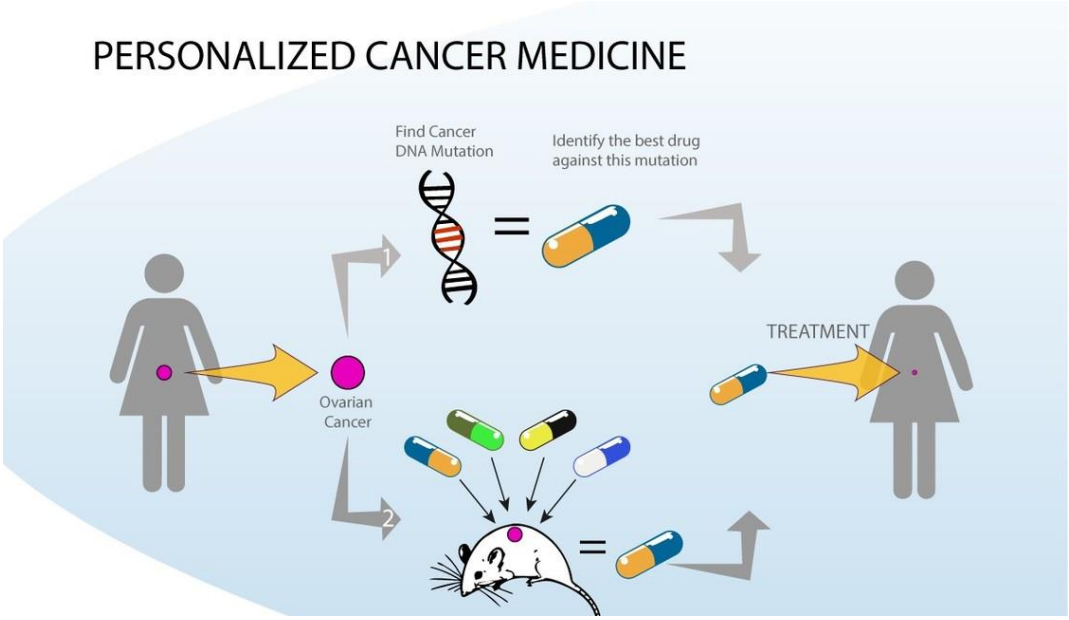


Fig-10 (Personalized medicine strstegies for cancer)

In summary, RNA-related diseases represent a heterogeneous group of disorders characterized by dysregulation of RNA molecules and RNA-protein interactions. Therapeutic strategies targeting RNA offer innovative approaches for treating these diseases, with ongoing clinical trials demonstrating the potential of RNA-targeting drugs in various disease settings. Personalized medicine approaches further enhance treatment efficacy by tailoring therapies to individual patients based on genetic, transcriptomic, and molecular characteristics, paving the way for precision medicine in the era of RNA-based therapeutics.

3. Challenges and Future Directions

A. Understanding Complex RNA-Protein Networks:

One of the major challenges in RNA biology is the elucidation of complex RNA-protein networks and their functional implications in health and disease. RNA molecules interact with a myriad of RNA-binding proteins (RBPs) in dynamic and context-dependent manners, forming intricate regulatory networks that govern diverse cellular processes. However, deciphering the specificity, dynamics, and regulatory mechanisms of RNA-protein interactions remains a daunting task. Future research efforts should focus on integrating experimental and computational approaches to systematically map RNA-protein interaction networks, characterize RNA-binding domains and motifs, and elucidate the regulatory roles of RBPs in cellular pathways. Advances in high-throughput sequencing, proteomics, and structural biology techniques will facilitate comprehensive profiling of RNA-protein interactomes, providing insights into the complexity and functional diversity of RNA-mediated processes.

B. Developing Targeted Therapies with Enhanced Specificity:

Another critical challenge in RNA-targeted therapeutics is the development of therapies with enhanced specificity and selectivity for disease-relevant RNA molecules or RNA-protein interactions. Traditional drug discovery approaches often face limitations in achieving precise targeting of RNA-mediated pathways, leading to off-target effects and suboptimal therapeutic outcomes. Future directions in drug development should prioritize the design of targeted therapies that selectively modulate disease-associated RNA targets while sparing normal cellular processes. This requires innovative strategies for rational drug design, including structure-based design of small molecule inhibitors, rational engineering of therapeutic oligonucleotides, and optimization of delivery systems for targeted drug delivery. Integration of computational modeling, highthroughput screening, and biomarker-driven approaches will facilitate the discovery of novel RNA-targeting drugs with enhanced specificity and therapeutic efficacy.

C. Translating Research Findings into Clinical Practice:

Translating research findings from bench to bedside represents a significant challenge in the field of RNA biology and therapeutics. Despite promising preclinical studies and early-phase clinical trials, many RNAtargeting drugs face hurdles in achieving regulatory approval and clinical adoption. Challenges in clinical translation include ensuring safety, efficacy, and scalability of RNA-based therapies, optimizing drug delivery and formulation strategies, and addressing regulatory and reimbursement considerations. Future directions in translational research should prioritize the development of robust preclinical models, validation of biomarkers for patient stratification, and implementation of adaptive clinical trial designs to expedite the evaluation of RNAtargeting drugs. Collaborative partnerships between academia, industry, and regulatory agencies are essential for navigating the regulatory pathway, securing investment, and overcoming barriers to clinical implementation. Ultimately, successful translation of RNAbased therapeutics into clinical practice holds the potential to revolutionize disease treatment and improve patient outcomes in diverse medical conditions.

In conclusion, addressing the challenges and future directions in RNA biology and therapeutics requires interdisciplinary collaboration, technological innovation, and translational research efforts. By advancing our understanding of complex RNA-protein networks, developing targeted therapies with enhanced specificity, and overcoming barriers to clinical translation, we can harness the therapeutic potential of RNA-targeting drugs and pave the way for precision medicine in the era of RNA-based therapeutics.

4. CONCLUSION

In conclusion, the study of RNA-protein interactions and the development of RNA-targeted therapeutics represent exciting frontiers in biomedical research with profound implications for disease treatment and precision medicine. RNA molecules play pivotal roles in cellular processes, and dysregulation of RNA-protein interactions underlies a wide range of diseases and disorders. The advancements in understanding the structural basis, dynamics, and functional implications of RNA-protein complexes have fueled the development of novel therapeutic strategies, including small molecule inhibitors, antisense oligonucleotides, RNA aptamers, and RNA splicing modulators. These RNA-targeting therapeutics offer promise for addressing unmet medical needs in various disease contexts, from cancer and genetic disorders to infectious diseases and neurological conditions. Furthermore, personalized medicine approaches leveraging insights into RNA biology, genetic variation, and molecular biomarkers hold the potential to tailor treatment strategies to individual patients, optimizing therapeutic efficacy and minimizing adverse effects. Despite the challenges in understanding complex RNA-protein networks, developing targeted therapies with enhanced specificity, and translating research findings into clinical practice, collaborative efforts across academia, industry, and regulatory agencies continue to drive innovation and accelerate progress in the field. By addressing these challenges and embracing future directions in RNA biology and therapeutics, we can harness the therapeutic potential of RNA-based interventions and usher in a new era of precision medicine for the benefit of patients worldwide.

REFERENCES

1. Wu P. Inhibition of RNA-binding proteins with small molecules. Nature Reviews Chemistry. 2020 Sep;4(9):441-58.
2. Fabbiano F, Corsi J, Gurrieri E, Trevisan C, Notarangelo M, D'Agostino VG. RNA packaging into extracellular vesicles: An orchestra of RNA-binding proteins?. Journal of extracellular vesicles. 2020 Dec;10(2):e12043.
3. Sun W, Cui H, Xu T, Yue J, Liang J, You W, Sun W, Yi Q. RNA binding proteins in extracellular vesicles and their potential value for cancer diagnosis and treatment. International Journal of Oncology. 2023 Oct 1;63(4):1-4.
4. Comunale BA, Larson RJ, Jackson-Ward E, Singh A, Koback FL, Engineer LD. The Functional Implications of Broad Spectrum Bioactive Compounds Targeting RNA-Dependent RNA Polymerase (RdRp) in the Context of the COVID-19 Pandemic. Viruses. 2023 Nov 25;15(12):2316.
5. Maurel MC, Biard B, Moulinier C, Braz D, Nugier J, Chaumas I, Reboud-Ravaux M, Décout JL. RNA-acting antibiotics: in-vitro selection of RNA aptamers for the design of new bioactive molecules less susceptible to bacterial resistance. Journal of pharmacy and pharmacology. 2002 Aug;54(8):1019-31.
6. Bertoldo JB, Müller S, Hüttelmaier S. RNA-binding proteins in cancer drug discovery. Drug discovery today. 2023 Apr 7:103580.

7. Denaro M, Smeriglio A, Barreca D, De Francesco C, Occhiuto C, Milano G, Trombetta D. Antiviral activity of plants and their isolated bioactive compounds: An update. Phytotherapy Research. 2020 Apr;34(4):742-68.
8. Kudinov AE, Karanicolas J, Golemis EA, Boumber Y. Musashi RNA-binding proteins as cancer drivers and novel therapeutic targets. Clinical Cancer Research. 2017 May 1;23(9):2143-53.
9. Lee SH, Shin H. Matrices and scaffolds for delivery of bioactive molecules in bone and cartilage tissue engineering. Advanced drug delivery reviews. 2007 May 30;59(4-5):339-59.
10. Venthur H, Zhou JJ. Odorant receptors and odorant-binding proteins as insect pest control targets: a comparative analysis. Frontiers in physiology. 2018 Aug 24;9:382769.
11. Fan Y, Dong D, Li Q, Si H, Pei H, Li L, Tang B. Fluorescent analysis of bioactive molecules in single cells based on microfluidic chips. Lab on a Chip. 2018;18(8):1151-73.
12. Suwannarach N, Kumla J, Sujarit K, Pattananandecha T, Saenjum C, Lumyong S. Natural bioactive compounds from fungi as potential candidates for protease inhibitors and immunomodulators to apply for coronaviruses. Molecules. 2020 Apr 14;25(8):1800.
13. Arjmand F, Yasir Khan H, Tabassum S. Progress of Metal-Based Anticancer Chemotherapeutic Agents in Last two Decades and their Comprehensive Biological (DNA/RNA Binding, Cleavage and Cytotoxicity Activity) Studies. The Chemical Record. 2023 Mar;23(3):e202200247.
14. Milcovich G, Lettieri S, Antunes FE, Medronho B, Fonseca AC, Coelho JF, Marizza P, Perrone F, Farra R, Dapas B, Grassi G. Recent advances in smart biotechnology: Hydrogels and nanocarriers for tailored bioactive molecules depot. Advances in colloid and interface science. 2017 Nov 1;249:163-80.
15. Titov DV, Liu JO. Identification and validation of protein targets of bioactive small molecules. Bioorganic & medicinal chemistry. 2012 Mar 15;20(6):1902-9.
16. Naidu SA, Mustafa G, Clemens RA, Naidu AS. Plant-derived natural non-nucleoside analog inhibitors (NNAIs) against RNA-dependent RNA polymerase complex (nsp7/nsp8/nsp12) of SARS-CoV-2. Journal of Dietary Supplements. 2023 Mar 4;20(2):254-83.
17. Morishita EC. Discovery of RNA-targeted small molecules through the merging of experimental and computational technologies. Expert Opinion on Drug Discovery. 2023 Feb 1;18(2):207-26.
18. Warner KD, Hajdin CE, Weeks KM. Principles for targeting RNA with drug-like small molecules. Nature reviews Drug discovery. 2018 Aug;17(8):547-58.
19. Angelbello AJ, Chen JL, Disney MD. Small molecule targeting of RNA structures in neurological disorders. Annals of the New York Academy of Sciences. 2020 Jul;1471(1):57-71.
20. Mouffouk C, Mouffouk S, Mouffouk S, Hambaba L, Haba H. Flavonols as potential antiviral drugs targeting SARS-CoV-2 proteases (3CLpro and PLpro), spike protein, RNA-dependent RNA polymerase (RdRp) and angiotensin-converting enzyme II receptor (ACE2). European journal of pharmacology. 2021 Jan 15;891:173759.
21. De Simeis D, Serra S. Actinomycetes: A never-ending source of bioactive compounds—An overview on antibiotics production. Antibiotics. 2021 Apr 22;10(5):483.
22. Jaiswal AK, Thaxton ML, Scherer GM, Sorrentino JP, Garg NK, Rao DS. Small molecule inhibition of RNA binding proteins in haematologic cancer. RNA biology. 2024 Dec 31;21(1):1-4.
23. Yi Q, Deng Z, Yue J, He J, Xiong J, Sun W, Sun W. RNA binding proteins in osteoarthritis. Frontiers in Cell and Developmental Biology. 2022 Aug 8;10:954376.

24. Řezanka T, Sigler K. Biologically active compounds of semi-metals. Studies in natural products chemistry. 2008 Jan 1;35:835-921.
25. Scassellati C, Galoforo AC, Bonvicini C, Esposito C, Ricevuti G. Ozone: a natural bioactive molecule with antioxidant property as potential new strategy in aging and in neurodegenerative disorders. Ageing Research Reviews. 2020 Nov 1;63:101138.
26. Remali J, Aizat WM. A review on plant bioactive compounds and their modes of action against coronavirus infection. Frontiers in pharmacology. 2021 Jan 11;11:589044.
27. Antonopoulou I, Sapountzaki E, Rova U, Christakopoulos P. Inhibition of the main protease of SARS-CoV-2 (Mpro) by repurposing/designing drug-like substances and utilizing nature's toolbox of bioactive compounds. Computational and Structural Biotechnology Journal. 2022 Jan 1;20:1306-44.
28. Young S, Wong M, Tabata Y, Mikos AG. Gelatin as a delivery vehicle for the controlled release of bioactive molecules. Journal of controlled release. 2005 Dec 5;109(1-3):256-74.
29. Ottoz DS, Berchowitz LE. The role of disorder in RNA binding affinity and specificity. Open Biology. 2020 Dec 23;10(12):200328.
30. Venturella G, Ferraro V, Cirlincione F, Gargano ML. Medicinal mushrooms: bioactive compounds, use, and clinical trials. International journal of molecular sciences. 2021 Jan 10;22(2):634.
31. Pan MH, Lai CS, Dushenkov S, Ho CT. Modulation of inflammatory genes by natural dietary bioactive compounds. Journal of agricultural and food chemistry. 2009 Jun 10;57(11):4467-77.
32. Feng YL, Chen DQ, Vaziri ND, Guo Y, Zhao YY. Small molecule inhibitors of epithelial-mesenchymal transition for the treatment of cancer and fibrosis. Medicinal research reviews. 2020 Jan;40(1):54-78.
33. Oskar M, Buchwald W, Nawrot R. Plant defense responses against viral and bacterial pathogen infections. Focus on RNA-binding proteins (RBPs). Herba Polonica. 2015;60(4):60-73.
34. Su X, Kong L, Lei X, Hu L, Ye M, Zou H. Biological fingerprinting analysis of traditional Chinese medicines with targeting ADME/Tox property for screening of bioactive compounds by chromatographic and MS methods. Mini reviews in medicinal chemistry. 2007 Jan 1;7(1):87-98.
35. Bouyahya A, Bakrim S, Aboulaghras S, El Kadri K, Aanniz T, Khalid A, Abdalla AN, Abdallah AA, Ardianto C, Ming LC, El Omari N. Bioactive compounds from nature: Antioxidants targeting cellular transformation in response to epigenetic perturbations induced by oxidative stress. Biomedicine & Pharmacotherapy. 2024 May 1;174:116432.
36. Deschamps AM, Spinale FG. Pathways of matrix metalloproteinase induction in heart failure: bioactive molecules and transcriptional regulation. Cardiovascular Research. 2006 Feb 15;69(3):666-76.