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ROLE OF NON-CODING RNAS IN POST-TRANSCRIPTIONAL REGULATION OF CELLULAR PROCESSES

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ABSTRACT

Non-coding RNAs (ncRNAs) have become an important regulator of post-transcriptional gene expression with varied molecular mechanisms that control diverse cellular functions. This literature review seeks to explain the functional role of major classes of ncRNA, such as microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) in the regulation of mRNA stability, translation, and degradation. A thorough literature based review of the latest discoveries was done to address the biogenesis, regulatory association, and functional roles of such ncRNAs and their connections in cellular pathways. The results show that the ncRNAs have a specific and dynamic regulation of gene expression by interplaying with target mRNAs and protein complexes, and have influence on cell proliferation, cell differentiation, cell apoptosis, and stress-response. In addition, it has been firmly linked that the ncRNAs networks are dysregulated in the pathogenesis of different diseases, such as cancer, neurological issues, and cardiovascular diseases. To sum up, ncRNAs are a unique level of post-transcriptional control, which promises much in terms of diagnostic biomarkers and therapeutic drugs of modern molecular medicine.

KEYWORDS: Non-coding RNA; miRNA; lncRNA; circRNA; Post-transcriptional regulation; Gene expression

1. INTRODUCTION

The process of gene expression regulation is essential to provide a very specific control of cellular functioning, development, and homeostasis. Conventionally, the concept of gene regulation used to be interpreted in the context of the central dogma, in the direction of genetic information flowing out of the DNA into the RNA and eventually into the protein. Although transcriptional regulation is central to shaping the amount of gene expression, growing data points to the significance of post-transcriptional signals in the process of refining the dynamics of gene expression (Statello et al., 2021). These regulation layers are crucial towards fast cellular responses to environmental stimulus and physiological stability in a variety of biological systems.

The post-transcriptional regulation is a multi-faceted process that involves mRNA splicing, transport, stability, and localization as well as translation efficiency. The entire processes define mRNA molecule fate and consequently affect protein synthesis without changing the genomic code. The non-coding RNAs (ncRNAs) is one of the most important regulators of these processes and represents a wide type of RNA molecules that lack a specific encoded protein but perform regulatory roles at different levels of gene activity (Chen & Kim, 2024). Specifically, microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) have been widely investigated in terms of their contribution to the regulation of mRNA stability and translation by means of sequence-specific interactions with their targets and complex regulation networks (Jonas & Izaurralde, 2015; Li et al., 2

MicroRNAs normally have their action in binding to complementary sequences on target mRNAs, resulting in translational repression or mRNA degradation, and thus are the major regulators of gene silencing pathways (O'Brien et al., 2018). Long non-coding RNAs have varied mechanisms, such as being molecular scaffolds,

guides, or decoys, chromatin remodelling and post-transcriptional controls (Kopp & Mendell, 2018). Circular RNAs have received interest due to their stability and by being able to act as microRNA sponges therefore indirectly control gene expression (Hansen et al., 2013; Memczak et al., 2013). Collectively, these groups of ncRNAs constitute complex regulatory networks that facilitate the coordination of the expression of genes in cellular activities.

Although considerable progress was made in the study of the functions of single ncRNAs, there is still a lack of an in-depth analysis of coordinated functions of these ncRNAs in post-transcriptional regulation. Isolated types of ncRNA or a particular signaling pathway are frequently studied, but the complexity of cross-talk and cross-network-based interactions including competing endogenous RNA (ceRNA) is ignored (Salmena et al., 2011; Tay et al., 2014). Such a fragmented conception leaves a very important gap in the comprehension of the overall regulation of cellular processes of proliferation, apoptosis, differentiation, and stress response by ncRNAs, whether in physiological or pathological physiological conditions.

Moreover, dysfunction of ncRNA networks has been closely connected with numerous illnesses, such as cancer, nervous system disorders, and cardiovascular diseases, and thus their clinical utility as biomarkers and therapy targets should not be underestimated (Anastasiadou et al., 2018). Nevertheless, there exist difficulties in applying these results to clinical practice because of the problems of behavior under condition and the redundancy in functions and the full characterization of ncRNA interaction networks.

Although there has been extensive advancement on the characterization of individual non-coding RNA (ncRNA) classes, there has not been a comprehensive and integrated knowledge on the coordinated functions of the non-coding RNA on post-transcriptional regulation. The majority of the available literature is still largely concentrated on individual mechanisms of microRNAs, long non-coding RNAs, or circular RNAs, and does not sufficiently discuss their interdependence of regulatory networks or cross-talk mechanisms, including competing endogenous RNA (ceRNA) interactions. Also, lack of system-level view on the overall contribution of these ncRNA networks in complex cellular processes, such as proliferation, apoptosis, differentiation and stress responses. Absence of cohesive models, combinative interactions of regulations in multi-layers, contextual variability and redundancy of functional aspects remain as impediments to converting ncRNA-based findings into clinically applicable endeavors.

The current review brings a holistic and integrative study of non-coding RNAs in post-transcriptional gene regulation by synthesizing the functions of the prominent classes of ncRNAs, such as miRNAs, lncRNAs, and circRNAs, in the context of a single framework. It stresses the interrelationship between ncRNA-mediated regulatory networks, including cross-talk, i.e. ceRNA interactions, and their effects on the control of gene expression. As well, the review provides a connection between molecular activities and functional results as the ncRNA activity is associated with the main cellular activities and pathogenesis of diseases. This publication gives important information regarding the possibilities of ncRNAs as diagnostic biomarkers and therapeutic targets as well as provides current trends of development of the research in the field of molecular and translational medicine.

2. CLASSIFICATION OF NON-CODING RNAs

Non-coding RNAs (ncRNAs) make up an important and functionally diverse collection of RNA molecules which are non-protein-coding, yet are critical in the regulation of gene expression, especially at the post-transcriptional scale. NcRNAs can be categorized in large groups based on their size, structure, and functional mechanisms, which are microRNAs (miRNAs), long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), and other small regulatory RNAs. MicroRNAs are short endogenous RNA molecules; on average, 20-24 nucleotides long, which regulate gene expression by binding their targets via sequence specificity with the target mRNA. Their formation is a multistage process that starts with the transformation of primary miRNAs, the processing of which into precursor miRNAs by the DroshaDGCR8 complex and their cleavage into mature miRNAs by Dicer in the cytoplasm (O'Brien et al., 2018). These developed miRNAs are integrated into the RNA-induced silencing complex (RISC) whereby they direct the complex to complementary mRNA targets with the result of mRNA degradation or translational repression based on the magnitude of complementarity (Jonas & Izaurralde, 2015).

Long non-coding RNAs on the other hand are characterised as transcripts of RNA which are longer than 200 nucleotides, have significant structural and functional diversity. All these molecules have the ability to bind the DNA, RNA and proteins and therefore they are able to regulate the expression of the genes in a number of different ways. LncRNAs are molecular scaffolds, which help to assemble protein complexes, guides, which help to recruit chromatin-modifying enzymes to particular genomic loci, and decoys, which help to recruit regulatory molecules like transcription factors or microRNAs (Kopp and Mendell, 2018; Statello et al., 2021). This is further established by their capacity to act in the nuclear and cytoplasmic compartments, which make them more regulatory versatile in the regulation of the expression of genes at a wide range of levels.

The other unique category of ncRNAs is the circular ones, which are covalently closed loop structures in which non-canonical splicing process is implemented (back-splicing). Such special design will increase the stability of circRNAs by avoiding degradation caused by exonuclease (Li et al., 2018). CircRNAs have been well known to serve a function of microRNA sponges, in which they couple to and trap miRNAs, in this way avoid interaction between them and their target mRNAs indirectly regulate gene expression (Hansen et al., 2013; Memczak et al., 2013). The mechanism helps the complex regulatory networks to be formed which modulate the cellular signaling pathways and physiological processes.

Besides these large ncRNAs, there are other ncRNAs of significance in the regulation of genes including small interfering RNAs (siRNAs), Piwi-interacting RNAs (piRNAs) and small nucleolar RNAs (snoRNAs). SiRNAs play a role in RNA interference pathways, which entail sequence-specific degradation of target mRNAs, whereas the major role of the piRNA is the silencing of transposons and genome stability in germline cells. The snoRNAs are associated with the chemical modification and processing of the ribosomal RNAs and, thus, the ribosome biogenesis (Palazzo & Lee, 2015). It is also emerging that these ncRNAs are also involved in wider regulatory networks, such as epigenetic regulation, RNA editing and cross-talk with other classes of ncRNA, suggesting that they are increasingly important regulators of cells. Together, all the different types of ncRNAs make up a complex regulatory network that allows the regulation of the gene expression to an exact degree and keeps the cellular homeostasis.

3. MECHANISMS OF POST-TRANSCRIPTIONAL REGULATION

Post-transcriptional regulation is an important gene regulation process, where non-coding RNAs (ncRNAs) regulate the destiny of the messenger RNAs (mRNAs) once they have been transcribed. The regulation of mRNA stability is considered one of the key mechanisms which is achieved by controlled pathways of RNA degradation. The Half-life of mRNA is determined by cellular processes that include exonucleolytic degradation and deadenylation-associated decay which in turn affect protein production. NcRNAs, especially microRNAs (miRNAs) and some long non-coding RNAs (lncRNAs) are able to either induce mRNA degradation or stabilize the transcript by binding to certain sequence elements and hence fine-tuning gene expression to cellular conditions. As shown in Fig 1 (mRNA Decay panel), miRNAs or lncRNAs interact with the target mRNAs and recruit exonucleases resulting in the progressive degradation of the transcript. This process is relevant to the quick suppression of the gene expression and is particularly typical during the stress or pathological responses.

Besides regulating the stability of mRNA, ncRNAs are key regulators of translation, by having an effect on the recruitment and activity of the ribosome. The ncRNAs can prevent or improve translation efficiency by a direct binding or interaction with the target mRNAs or by the association with translation initiation factors. The Translational Repression panel in Fig 1 shows that the ncRNAs disrupt the assembly of ribosome on mRNA which essentially prevents protein synthesis. MiRNAs in most cases interact with the 3 5 un-translated region (UTR) of mRNAs, and this leads to inhibition of translational events and does not necessarily lead to the degradation of the mRNA. On the other hand, some lncRNAs are able to facilitate the translation by stabilizing the ribosome-mRNA interaction, which suggests the dual regulatory nature of ncRNAs.

One of the key processes of post-transcriptional regulation is the RNA interference (RNAi) pathway that is mainly worked out by the RNA-induced silencing complex (RISC). The conventional miRNA loading of the Argonaute proteins in the RISC complex is an active silencing unit that is shown in RNA Interference (RNAi) panel of Fig 1. The complex itself is then directed to complementary target mRNAs by sequence- specific recognition. Based on the degree of complementarity, the RISC complex will cause direct mRNA cleavage or translation repression which is a gene silencing process. The pathway is an extremely conserved one that is important in ensuring gene expression faithfulness and plays an essential role in cellular development, immune regulation and differentiation. Moreover, ncRNAs are also involved in RNA editing and splicing modulation which increases complexity of regulation. These cause an effect on splicing alternative splicing by binding to splicing equipment and pre-mRNA transcripts thus producing various protein isoforms of one gene. Fig 1 shows RNA Modification panel that is important to recognize the functions of epitranscriptomic modifications like N6-methyladenosine (m6A) that controls RNA stability, processing, and translation. NcRNAs are becoming known to be engaged in the regulation of these adjustments and hence connect the processes of RNA editing with the processes of post-transcriptional regulation. The figure is used to explain how the m6A marks affect the alternative splicing events and subsequent RNA processing pathways which in turn affect the gene expression.

Together the four panels that are interrelated in Fig 1 give a very detailed visualization of post-transcriptional regulatory mechanisms mediated by ncRNAs, such as degradation of mRNA, translational repression, RNA interference through RISC complex, and RNA modification dependent regulation. These signaling pathways interact in a well-coordinated way to form accurate and dynamic regulation of gene expression, which highlights the key importance of ncRNAs to cell functioning and homeostasis.

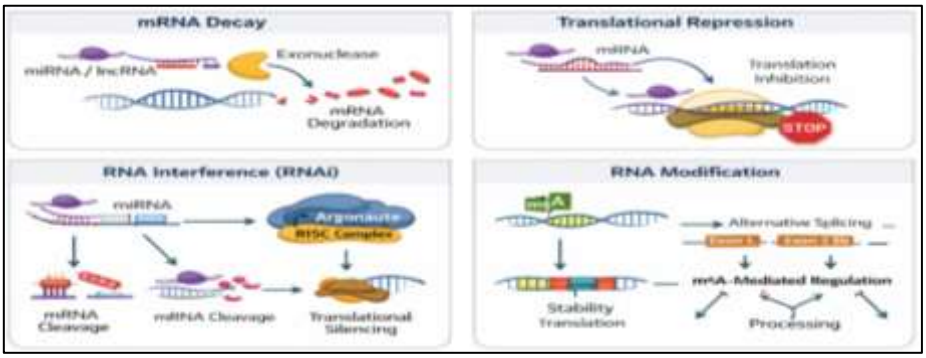

Fig 1. NcRNA-Mediated Post-Transcriptional Regulation Mechanisms.

4. NcRNAs IN CELLULAR PROCESSES

ncRNAs are non-coding RNAs that are major in the regulation of the crucial cellular functions through the post-transcriptional regulation of gene expression. Their role in the process of cell cycle control is one of the most significant functions of ncRNAs, and they do impact the expression and the activity of cyclins and cyclin-dependent kinases (CDKs), which play a crucial role in the process of cell cycle. The translation of cyclin transcripts can be suppressed by the action of MicroRNAs (miRNAs) and inhibit cell proliferation, whereas some long non-coding RNAs (lncRNAs) can boost cell cycle progression either by stabilizing mRNAs or by interacting with transcriptional regulators. Fig 2 (Cell Cycle Regulation module) shows that ncRNAs have direct interactions with cyclin/CDK regulatory loops and affect the transition between major phases of the cell cycle before, during, and after G1, S and G2, maintaining the cell division under the control and inhibiting inappropriate proliferation. NcRNAs are also major regulators of cell survival pathways, including apoptosis, in addition to controlling the cell cycle. They also have an interaction with critical signaling molecules like BCL-2 family proteins and tumor suppressor p53, and therefore they regulate the equilibrium between pro-apoptotic signals and anti-apoptotic signals. In Fig 2 Apoptosis and Cell Survival module, miRNAs and antisense RNAs have been shown to regulate the p53 signaling and BCL-2 expression with the switch to either the survivor or programmed cell death. This regulatory balance is essential in getting rid of unhealthy cells and keeping the tissues intact and its failure to do so is closely related to tumor development and progression of diseases.

It is also through the NcRNAs that cellular differentiation and cellular development occurs; they are known to regulate the cell fate-determining gene expression programs. The expression of transcription factors that regulate self-renewal and lineage specification are regulated by ncRNAs in stem cells. Fig 2 Differentiation and Development module shows the process in which the stem cells differentiate into tissue-specific cells, which is mediated by the ncRNA-controlled gene expression networks. This involves signaling pathway modulation which drives differentiation as well as connecting apoptotic pathways to developmental processes of remodeling. This regulation will provide appropriate tissue formation and specialization of functions.

Moreover, ncRNAs actively participate in the cellular stress responses and cellular homeostasis. During the oxidative stress, ncRNAs are able to control the antioxidant defense and regulate the stress-responsive gene expressions. The Stress Response and Homeostasis module in Fig 2 indicates the effects that ncRNAs have on reactive oxygen species (ROS) signaling, cytokine production, and immune cell activation. These processes allow ncRNAs to make cells more adaptive to environmental stressor, as well as to physiological balance. The combined architecture of Fig 2, with ncRNAs as a regulatory node, proves the role of these molecules in coordinating several events in a cell at the same time. The cyclic structure of the pathways in the figure highlights the interdependence of the cell cycle progression, apoptosis, differentiation and stress response, which ncRNAs are not independent regulators but important parts of an interconnected and dynamic cellular regulatory system.

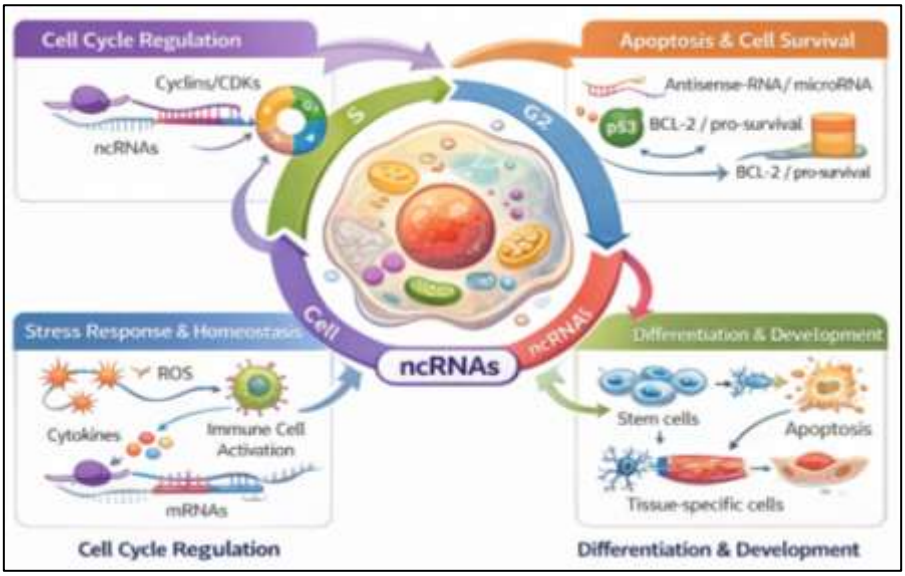

Fig 2. NcRNA Regulation of Cellular Processes.

5. ROLE OF ncRNAs IN HUMAN DISEASES

The fact that non-coding RNAs (ncRNAs) are post-transcriptional regulators has made them critical to the pathogenesis of numerous human diseases in that they have the capacity to regulate the expression of genes. The ncRNAs act as oncogenes or tumor suppressors in cancer in relation to their targets and context of regulation. OncomiRs, like miR-21, stimulate tumor progression through down regulation of tumor suppressor miRNAs and inhibit proliferation and induce apoptosis through down regulation of oncogenic pathways (tumor-suppressive ncRNAs). HOTAIR is a long non-coding RNA that plays a role in tumorigenesis in the process of chromatin remodelling and metastasis, whereas circular RNAs (circRNAs) can regulate cancer progression by endogenous

competing RNA (ceRNA) interactions. More importantly, these ncRNAs are highly stable and tissue specific as shown in Table 1, and they can be used as significant biomarkers in the early detection of cancer, prognosis, and targeted therapy.

ncRNAs have been shown in neurological diseases to regulate neuron functioning and their plasticity at the synaptic end, and their maladjustment to disease pathogenesis, including Alzheimer, and Parkinson disease. In summary as seen in Table 1, miR-29 is a major player in the processing of amyloid precursor protein, hence, it affects the process of amyloid-beta in the Alzheimer disease. On the same note, lncRNA BACE1-AS regulates the expression of 2 secretase, which also leads to neurodegenerative pathology. These illustrations indicate the role played by ncRNAs in balancing key molecular pathways to guarantee neuronal survival, neuroinflammation, and synaptic signaling.

NcRNA are also important in cardiovascular diseases especially in cardiac remodelling, hypertrophy and fibrosis. According to Table 1, miR-133 plays the role of controlling cardiac hypertrophy through the action of the genes related to muscle development and lncRNA ANRIL actively participates in atherosclerosis and inflammation of the vessels. Abnormal regulation of these ncRNAs will also disrupt the normal cardiovascular signaling by causing structural and functional changes in cardiac tissue and ultimately events that result in disease progression.

The ncRNAs regulate the major pathways in glucose metabolism, insulin signaling, and lipid homeostasis in metabolic diseases, such as diabetes and obesity. As an example, miR-375 is important in the functioning of pancreatic 8-cells and insulin release, whereas circRNA circHIPK3 has an impact on the glucose metabolism and the growth of the cells, as indicated in Table 1. Moreover, lncRNAs also regulate adipogenesis and energy homeostasis, which also proves the extensive regulatory capabilities of ncRNAs in the process of metabolism.

Altogether, Table 1 gives a comparative and integrative summary of the role of ncRNA in different major disease categories and well demonstrates how particular ncRNAs are associated with particular pathological processes. The table brings out the diversity of ncRNA functions and at the same time shows their similarity in being key regulators of gene expression in disease conditions. The table supports the idea that ncRNAs are important molecular interconnectors between regulation of genes and pathogenesis of disease, providing a lot of potential in terms of diagnostic and therapeutic improvements.

Table 1. NcRNAs in Major Human Diseases and Their Functional Roles

Disease Category	ncRNA Type	Example	Functional Role
Cancer	miRNA	miR-21	Oncogenic (inhibits tumor suppressor genes)
Cancer	lncRNA	HOTAIR	Promotes metastasis and chromatin remodeling
Neurological Disorders	miRNA	miR-29	Regulates amyloid processing
Neurological Disorders	lncRNA	BACE1-AS	Modulates Alzheimer's pathology
Cardiovascular Diseases	miRNA	miR-133	Regulates cardiac hypertrophy
Cardiovascular Diseases	lncRNA	ANRIL	Involved in vascular inflammation
Metabolic Disorders	miRNA	miR-375	Controls insulin secretion
Metabolic Disorders	circRNA	circHIPK3	Regulates glucose metabolism

6. EXPERIMENTAL AND COMPUTATIONAL APPROACHES

Research and description of the non-coding RNAs (ncRNAs) are based on a complex of sophisticated experimental and computational methodology that allows to identify, validate, and analyze the functions of these regulatory molecules. Most of them include high-throughput RNA sequencing (RNA-seq), which has proven to be a very potent instrument of performing a complete profile of the transcriptome that enables the identification of known and novel ncRNAs in varying biological conditions. RNA-seq offers the quantitative information of the patterns of expression, alternative splicing, and transcript diversity, and thus, opens up the possibility of exploring ncRNA-mediated regulatory networks. As shown in Fig 3 (RNA Sequencing module), this step entails the isolation of RNA samples and sequencing as well as producing large scale datasets which form the basis of input to the downstream analysis and experimental procedures.

In order to confirm the profiles of the expression observed in studies involving sequencing, reverse transcription quantitative polymerase chain reaction (RT-qPCR) is commonly used because it is highly sensitive and specific. Fig 3: RT-qPCR Validation module represents a graphical representation of the process in which the amplification-based techniques applied to the selected ncRNA candidates identified with the help of the RNA-seq are compared with their quantitative results. This would confer accuracy, reproducibility and reliability of expression data, especially getting validation of the patterns of differential expression and identification of the possible biomarkers.

Besides the analysis of expression, the functional characterization of ncRNAs is also possible with the help of the genome-editing technologies, e.g. CRISPR-based systems. As depicted in the CRISPR Functional Studies module of Fig 3, CRISPR-Cas platforms can be applied to specific ncRNA loci to allow the researcher to study their biological functions by knocking out, activating, or mutating the gene. This is essential in determining causal relationships between ncRNA and cell phenotypes in that it will further improve our knowledge about their regulatory roles in normal physiology and in disease events.

Bioinformatics tools are a complement to the field of ncRNA analysis and interpretation, which is predominantly carried out in line with experimental methods. Fig 3 Computational Analysis module indicates the process of

sequencing outputs in pipelines, which include sequence alignment, transcript assembly, the differential expression analysis, and target prediction. The more sophisticated computational tools also facilitate the network construction such as ncRNA-mRNA interaction mapping and competing endogenous RNA (ceRNA) network analysis. These methods combine multi-omics data sets to give a systems view of the ncRNA-mediated regulation. Lastly, the lower panel of Fig 3 (Downstream Integration module) is the assimilation of experimental and computational discoveries into biologically useful knowledge. These involve pathway analysis, functional annotation and regulatory network identification between ncRNAs and certain cellular processes and disease mechanisms. Fig 3 has an organised and linked design that favours a lean workflow, discovery, RNA-seq, validation, RT-qPCR, functional characterization, CRISPR, and computational interpretation, which explains how the multidisciplinary strategy works together to contribute to ncRNA studies.

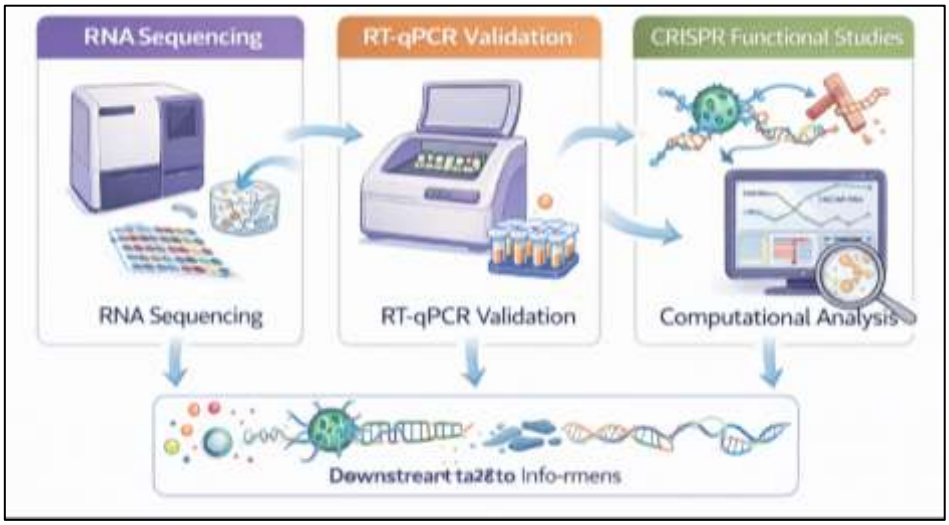

Fig 3. NcRNA Identification and Validation Workflow.

7. DISCUSSION

The discoveries made in the present review have shown the combined action of various non-coding RNAs (ncRNAs) classes of microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) in coordinating post-transcriptional gene regulation. These ncRNAs do not act in isolation but instead as members of regulatory networks, in which cross-talk processes, including the presence of competing endogenous RNA interactions allow them to act upon gene expression in a coordinated manner. With the use of this network-based regulation, the ncRNAs are capable of simultaneously affecting numerous molecular pathways, and hence controlling key cell processes including proliferation, apoptosis, differentiation, and stress response. The high specificity and multi-target capacity of ncRNA-mediated regulation is a strong characteristic of this regulation method. Single miRNAs are capable of controlling a large number of downstream transcripts, and in many cases the miRNA covers whole signaling pathways and no single genes. Similarly, lncRNAs and circRNAs act as scaffolds, guides, decoys, or sponges, which increases the scope of their regulatory actions through the post-transcriptional control. Nevertheless, this complexity is also associated with complications such as functional redundancy, overlapping of pathways and high context-dependence of tissues and disease conditions.

Fig 4 here offers a significant bibliometric insight into the fast growth of the research on ncRNA in the course of time. The graph shows a continuous rise in the number of ncRNA-related publications from 5,000 in 2000 to 30,000 in 2010, reaching 65,000 in 2018 and approximately 95,000 in 2024. Mathematically, this is almost a 19 fold rise in the 24 years period. The increase from 2000 to 2010 alone corresponds to a 500% rise, while the growth from 2010 to 2018 is approximately 116.7%, and the rise from 2018 to 2024 is about 46.2%. These values suggest not only the continued penetration of the interest in research but also show the expedition of scientific interest in the past decade, which is a strong testimony to the increased awareness of ncRNAs as significant activity regulators influencing the nature of cellular functions and disease processes.

On further analyzing Fig 4, it can be interpreted that the field went through several phases. The initial period between 2000 and 2007 indicates that the growth is rather slow-paced, and the number of publications is growing between 5,000 and approximately 18,000, which is the first period when miRNAs were the center of interest. The period between 2008 and 2016 is steeper and the output of the field has increased about 21,000 to 50,000, which corresponds to the wider acceptance of lncRNAs, circRNAs, and ceRNA networks. The last stage of 2017 to 2024 demonstrates further vigorous upward trend with a range of 55,000 to 95,000, which reflects the shift of the ncRNA research study out of the primary research of basic molecular biology into the research sectors of translational research oncology, neurodegeneration, cardiovascular biology, and biomarkers.

Notably, the dotted trend of Fig 4 indicates that the growth is not only linear as it is described by an acceleration trend, and it implies that the study on ncRNA is one of the fastest-growing fields in contemporary molecular medicine. This rapid change in the number of publications is an indirect argument that ncRNAs possess significant

scientific benefits, among which are the ability to regulate processes and have a pathway-wide impact, as well as be clinically relevant. Simultaneously, the graph also suggests that the field is becoming more complicated as the growth of publications has been in tandem with the increase in conflicting results, tissue-specific interpretations, and overlapping functional statements across the ncRNA groups.

This observation can be applied directly to the challenges that are discussed in this review. Despite the abundance of research showing high biological and clinical significance, this also represents an unresolved problem of redundancy in functions because in some cases, several ncRNAs are known to regulate similar targets, and cell type, disease, and developmental stage-specific behavior because in some cases the same ncRNA can behave differently. Therefore, the trend in Fig 4 is not only a manifestation of scientific expansion, but also the highlight of the rising necessity of standardized validation systems, comparative mechanistic investigations and integrative systems biology representations. Altogether, the growing body of literature presented in Fig 4 supports the main conclusion of this review, namely, that ncRNAs have become the focus of the transition of being viewed as adjunctive regulatory molecules to being obligate constituents of the control machines that regulate health and disease. Their benefits in specificity and multi-target regulation provide considerable appeal in both diagnostic and therapeutic purposes, whereas their redundancy and dependence on context is one of their biggest limitations that will have to be overcome before they can be widely applied in clinical settings.

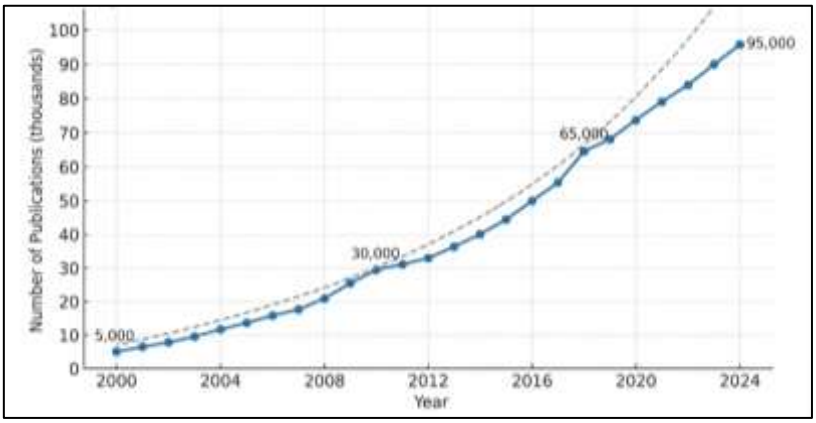

Fig 4. Trend of ncRNA Research Publications (2000–2024).

8. LIMITATIONS

Although there has been a tremendous development in the knowledge on non-coding RNA (ncRNA)-mediated post-transcriptional regulation, there are numerous drawbacks that limit the current level of research. One significant issue is that in vitro experiments and computational predictions are highly depended on, which, despite their usefulness, cannot be entirely useful in defining ncRNA behavior under physiological conditions. Most cell line research findings are not directly proven in the system of the living so their applicability to living organisms is limited. Additionally, the complexity and dynamism of the ncRNA interactions is another level of complexity because most ncRNA-target interactions can occur via parallel and interlinking pathways and it is not easy to determine specific functionality. Competing endogenous RNA (ceRNA) networks make apparent further complications on interpreting regulatory mechanisms since it forms cross-talk and feedback. The absence of standard datasets and analysis models to conduct ncRNA studies is yet another issue that poses a significant drawback as the research findings and results vary across studies, and this issue may cause problems with reproducibility. Variations in experimental conditions, sequencing platforms, and bioinformatics pipelines are some of the factors that result in discrepancy in the interpretation of data. All these restrictions point to the necessity of more powerful experimental validation, standardized procedures, as well as integrative strategies that will allow obtaining a complete picture of the ncRNA role in cellulose mechanisms and in diseases.

9. FUTURE PERSPECTIVES

The fast growing knowledge about the non-coding RNAs (ncRNAs) in post-transcriptional regulation of the genes provides potential opportunities of future research and clinical purposes. The most important avenue is the creation of ncRNA-based therapeutic, where microRNA mimics, antisense oligonucleotides, and small interfering RNAs could be developed to be able to control the expression of disease-related genes with a great level of specificity. These methods have a great potential in the treatment of complicated diseases, such as cancer, neurodegenerative conditions and metabolic syndromes. Simultaneously, the ncRNA profiling added into the systems of precision medicine may provide potential for the implementation of personalized diagnostics and targeted treatment because the ncRNA expression profiles could be regarded as highly sensitive and disease-specific bio-markers.

The discovery of ncRNA and application to functional characterisation is anticipated to accelerate further in the near future with further advances in artificial intelligence (AI) and machine learning. The models based on AI are capable of analyzing large volumes of transcriptomic and multi-omics data to discover new ncRNAs, make predictions, and create complicated regulatory networks with greater precision. Such types of computations will

be important towards eliminating the existing limitations in data complexity and interpretation. Nevertheless, regardless of these encouraging trends, there are numerous issues that still lie on the way to implementing the ncRNA research in the clinical practice. The problems like efficiency in the delivery, off-target effects, biological stability and variation in the patients group still remain the topics which hamper the implementation of therapeutic measures. Moreover, non-standardized clinical guidelines and safety long-term information is an obstacle to regulation and mass usage. These issues will be critical to NcRNA developing potential as a paradigm of molecular healthcare in the next generation through interdisciplinary studies, enhanced delivery models, and strong clinical validation.

CONCLUSION

The non-coding RNAs (ncRNAs) have become central regulators of post-transcriptional gene expression that is crucial in the process of regulating the stability of mRNA, translation, and degradation of mRNA in a variety of cellular activities. The ncRNAs play a significant role in the restoration of cellular homeostasis and the accurate regulation of biological processes, including proliferation, differentiation, apoptosis, and stress response, due to their capacity to interact with several molecular targets and be mediators of the very complicated regulatory mechanisms. Their dysregulation has been closely related with the broad spectrum of human ailments which makes them very crucial in physiological and pathological settings. In addition, the property of ncRNAs, such as their stability, specificity, and tissue-specific expression, highlighted the high potential of ncRNAs as a diagnostic biomarker and treatment targets. With the ongoing development of research, the incorporation of ncRNA-based approaches into clinical and translational systems is likely to have a paradigm shift in the next-generation molecular medicine by introducing a set of more specific, efficient, and personalized health-related solutions.

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