International Journal on Science and Technology (IJSAT)
E-ISSN: 2229-7677 ● Website: www.ijsat.org ● Email: editor@ijsat.org

The Emerging Role of Micro RNAs in Human Disease: A Review of Complete Understanding of Mechanism, Functions and Implications

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ABSTRACT

MicroRNAs (miRNAs) are pivotal regulators of gene expression, influencing a myriad of biological processes such as development, cell differentiation, and stress responses. These small, non-coding RNA molecules exert their effects by binding to complementary sequences on target messenger RNAs (mRNAs), leading to mRNA degradation or inhibition of translation. Recent advancements have illuminated the complexity of miRNA networks, revealing their role in orchestrating not only individual gene regulation but also broader signaling pathways. Dysregulation of miRNAs is increasingly recognized as a contributing factor in various diseases, including cancer, neurodegenerative disorders, and cardiovascular diseases. This project aims to elucidate the mechanisms by which miRNAs regulate cellular functions and their potential implications in disease pathology. By integrating bioinformatics analyses and experimental validation, we seek to identify key miRNA-mRNA interactions that could serve as therapeutic targets or biomarkers for precision medicine. Understanding these networks could pave the way for innovative approaches to diagnosis and treatment.

Keywords: MicroRNA, singling, Dysregulation, Bioinformatics, Cell – differentiation

1. INTRODUCTION

An intriguing example of tiny RNAs is MicroRNAs (miRNAs), a group that has an important role in gene regulation in a variety of biological processes and is involved in intracellular signaling. With a length of 22 nucleotides, these molecules are not passive participants in the cell, but rather dynamic ones that interact with target messenger RNAs (mRNAs) to precisely control gene expression [1]. Most often, this binding of mRNA results in loss of translation or destruction of the mRNA, thus regulating the assembly of proteins with extreme precision [2]. The biogenesis of miRNAs contains multiple essential phases which must occur in a precise order. To begin with, there is the formation of long primary transcripts, also known as pri-mRNAs, which are then subjected to processing by a microprocessor complex containing the RNase III known as Drosha. The process leads to the generation of the precursor miRNA (pre-miRNA) that is then taken to the cytoplasm, where it is cleaved by Dicer to form the mature miRNA duplexes. This duplex has one of its strands assembled onto the miRNA Induced Silencing Complex (miRISC) so that

the complex can be directed to its mRNA targets by the strand [3]. We cannot underestimate the evolutionary importance of miRNAs. These arose during the early stages of multicellularity and have since diversified greatly due to their importance in developmental and cytogenic processes [4]. This also means that miRNAs are crucial for the understanding of multiomics networks and have prospective biomedical implications, for instance, in oncology, and hereditary pathologies [5]. This complex relationship between miRNAs and gene expression illustrates the profound significance of these molecules as vital components of life's machinery.

CLASSIFICATION OF MICRO RNAs

MicroRNAs (miRNAs) can be categorized according to a number of factors, such as their structural features, functional roles, and biogenesis. You can use this special classification system in your research article

1. **Pathways of Biogenesis**

- **Canonical miRNAs**: The Drosha-Dicer pathway processes main transcripts to produce these most prevalent miRNAs. Usually, they show a well defined maturation process [6].
- **Mirtrons:** Without going through the Drosha processing stage, these are produced by splicing events of introns. They have unique structural characteristics and are frequently shorter [7].
- **Non-canonical miRNAs**: These are miRNAs that are produced by alternate processing processes or from other RNA species, for example, and do not follow the traditional biogenesis pathways [8].

2. **Functional Roles**

Oncogenic miRNAs, also known as OncomiRs, target tumor suppressor genes to encourage the growth of cancer. MiR-155 and miR-21 are two examples [9].

- **Tumor Suppressor miRNAs**: These are frequently downregulated in malignancies and block oncogenes. Let-7 and miR-34a are noteworthy examples [10].
- **Housekeeping miRNAs**: These, including miR-16 and miR-17, are crucial in preserving homeostasis and fundamental cellular processes [11].

3. **Structural Variants (IsomiRs)**

- **Canonical IsomiRs**: These may vary in length at either end but share the same sequence as the reference miRNA [12].
- **5′ IsomiRs**: Variants with a different length at the mature sequence's 5′ terminus [13].
- **3′ IsomiRs**: Variants with a different 3′ end length [12].
- **Polymorphic IsomiRs**: Variants having nucleotide variations within the sequence but the same overall length [13].
- **IsomiRs of the Mixed Type**: Variants that differ in both length and sequence [12].

4. **Expression Patterns**

- **Tissue-specific miRNAs**: These have an impact on regional biological processes and are mostly expressed in particular tissues or developmental stages [14].
- **Circulating miRNAs**: These miRNAs, which are present in physiological fluids like blood, may be used as biomarkers for a number of illnesses [15].

5. **Evolutionary Conservation**

- **Conserved miRNAs**: Showing important biological roles, these molecules are highly conserved across species [16].
- **Novel/Species-specific miRNAs**: These are recently discovered or particular to a given organism, and they are frequently linked to evolutionary adaptations [17].

Biogenesis Pathways of MicroRNAs

Small non-coding RNAs known as microRNAs (miRNAs) are essential for the control of genes. Canonical miRNAs, mirtrons, and non-canonical miRNAs are the three primary paths into which their biogenesis can be divided.

Canonical miRNAs

The canonical pathway is the predominant route for miRNA biogenesis. It involves several key steps:

1. **Transcription:** miRNA genes produce the primordial miRNA, or pri-miRNA [18].
2. **Drosha-mediated processing**: In the nucleus, the enzyme Drosha and the cofactor DGCR8 form the microprocessor complex, which cleaves the pri-miRNA to create precursor miRNA (pre-miRNA). Since it establishes the first maturation of miRNAs, this step is crucial [19].
3. **Export to Cytoplasm**: In a RanGTP-dependent way, Exportin 5 transports the premiRNA to the cytoplasm [20].
4. **Dicer Processing**: The pre-miRNA is further processed by Dicer in the cytoplasm to create a mature miRNA duplex, which is made up of the 5p and 3p strands [21].
5. **Formation of miRISC**: The miRNA-induced silencing complex (miRISC), which mediates translational repression of target mRNAs, is formed by loading one strand into the Argonaute (AGO) protein [22].

Mirtrons

Mirtrons represent an alternative pathway that bypasses some steps typical of canonical biogenesis:

- **Origin:** mRNA introns that are spliced out during RNA processing are the source of mirtrons [23].
- **Processing:** Dicer processes them after they are exported straight to the cytoplasm; this speeds up the maturation process because Drosha cleavage is not necessary [24]

- **Features**: Mirtrons have distinct structural characteristics that set them apart from normal pre-miRNAs, and they are often shorter than canonical miRNAs [23].

Non-Canonical miRNAs

Non-canonical pathways include various mechanisms that do not conform to the classical biogenesis model:

1. **Dicer-Independent Pathways**: Without Dicer's assistance, some miRNAs can be produced from other RNA species or by other processing techniques. For example, Drosha can digest short hairpin RNAs (shRNAs) and load them straight onto AGO proteins so they can mature [25].
2. **Diverse Origins:** This category highlights the adaptability and complexity of miRNA biogenesis by encompassing a variety of non-canonical miRNAs, such as those produced from snoRNAs or other non-coding RNAs [26].

Functional Roles of MicroRNAs in Cancer

Because they can function as tumor suppressors or oncogenes, microRNAs (miRNAs) are important players in the biology of cancer. Oncogenic miRNAs (oncomiRs), tumor suppressor miRNAs, and housekeeping miRNAs are among their functional categories.

1. **Oncogenic miRNAs (OncomiRs)** : OncomiRs are miRNAs that promote cancer development by targeting and inhibiting tumor suppressor genes. Key characteristics include:

Role in Tumorigenesis: They facilitate cancer progression by downregulating genes that normally suppress tumor growth [27].

Examples: miR-21: Frequently overexpressed in various cancers, it targets multiple tumor suppressors, contributing to cell proliferation and survival [28].

miR-155: Involved in immune response modulation and is often upregulated in hematologic malignancies, where it promotes oncogenic pathways [29].

2. **Tumor Suppressor miRNAs**

Tumor suppressor miRNAs function to inhibit oncogenes and are typically downregulated in cancerous tissues. Their roles include:

Inhibition of Oncogenes: They counteract the effects of oncomiRs, thus playing a protective role against tumor formation [30].

Examples: let-7: Known for its ability to target several oncogenes, its downregulation is linked to increased malignancy in various cancers [31].

miR-34a: Regarded as a crucial tumor suppressor due to its regulation by p53; it targets multiple oncogenes and is often found at low levels in tumors [32].

3. Housekeeping miRNAs

Housekeeping miRNAs are essential for maintaining basic cellular functions and homeostasis. These miRNAs are involved in various cellular processes and typically include:

Role in Cellular Function: They regulate fundamental biological processes such as cell cycle control and apoptosis [33].

Examples: miR-16: Plays a role in regulating cell proliferation and apoptosis, maintaining normal cellular functions [34].

miR-17: Involved in the regulation of cell growth and differentiation, contributing to overall cellular homeostasis [35].

Classification of IsomiRs

IsomiRs can be categorized into five distinct classes based on their structural characteristics:

1. **Canonical IsomiRs**: These are the standard miRNAs with sequences reported in miRNA databases. They serve as the reference for comparing other variants [36]. 2. **5′ IsomiRs**: Variants that differ in length at the 5′ end of the mature miRNA sequence. This alteration can affect the stability and targeting of the miRNA [37]. 3. **3′ IsomiRs**: These variants show differences in length at the 3′ end of the mature sequence, which may influence their interaction with target mRNAs [36].
4. **Polymorphic IsomiRs**: These maintain the same length as the canonical miRNA but have nucleotide variations within their sequences, which can lead to altered functionality [37].
5. **Mixed Type IsomiRs**: Variants that exhibit both length and sequence variations, potentially leading to unique regulatory roles distinct from their canonical counterparts [36].

Biological Relevance of IsomiRs

According to research, isomiRs have important functions in gene regulation and are not just byproducts of miRNA processing. They can target comparable mRNA networks and frequently exhibit expression patterns in common with canonical miRNAs, indicating a cooperative role in biological pathways [38]. For example, research has demonstrated that isomiRs can alter the activity of target genes implicated in important processes like the development of cancer and neurological illnesses [39].

Functional Implications

The presence of isomiRs introduces complexity into the miRNA landscape:

Target Redirection: In contrast to their conventional forms, isomyriRs may reroute binding to distinct mRNA targets, which could impact the results of gene expression. Certain isomiR variations, for instance,

have been demonstrated to either increase or decrease interactions with target genes, resulting in varying biological effects [40].

Clinical Significance: IsomiR dysregulation has been linked to a number of illnesses, including cancer, underscoring their potential as prognostic and diagnostic biomarkers . They are significant prospects for therapeutic interventions due to their capacity to alter gene regulatory networks [41]

Expression Patterns of miRNAs

The unique expression patterns of microRNAs (miRNAs) are essential for their functions in a variety of biological processes. Circulating miRNAs and tissue-specific miRNAs are two important types of miRNA expression.

Tissue-Specific miRNAs

- **Definition**: Tissue-specific miRNAs (TS miRNAs) impact local biological activities and processes by being primarily expressed in specific tissues or developmental stages [42]

- **Function in Development**: Tissue identity, differentiation, and function are all maintained by TS miRNAs. Rather than being limited to tissue-specific functions, they are engaged in the regulation of several signaling pathways. For instance, research has linked TS miRNAs to conditions like cancer, diabetes, and cardiovascular disease [43].

- **Expression Patterns**: The level of specificity for each miRNA in various tissues can be measured using a tissue specificity index (TSI). The values vary from 0 (which is expressed in multiple tissues) to 1 (which is expressed in just one tissue). As demonstrated by miR-133b and miR-206 in muscle tissues and other brain-specific miRNAs, high TSI values signify robust tissue-specific expression [44].

- **Regulatory Networks**: Recent research has revealed complex regulatory networks involving transcription factors (TFs) that interact with TS miRNAs across multiple tissues. These interactions help elucidate how TS miRNAs contribute to the physiological functions of different tissues [45].

Circulating miRNAs

- **Definition**: Circulating miRNAs can be used as possible biomarkers for a number of disorders and are present in biological fluids like blood [46].

- **Potential as a Biomarker**: Certain circulating miRNAs have been connected to certain clinical disorders, which makes them useful for non-invasive diagnosis. For example, it has been demonstrated that several isomiRs can distinguish between healthy and malignant tissues, suggesting that they may be used as tumor type indicators [47].

Circulating miRNAs can be attached to proteins or packaged in exosomes, which prevents them from degrading and makes it easier for them to move throughout the body. They can affect distant tissues and aid in intercellular communication thanks to this mechanism [48].

Evolutionary Conservation of miRNAs

Small non-coding RNAs known as microRNAs (miRNAs) are classified according to their evolutionary conservation and are essential for the control of genes. Knowledge of miRNAs' conservation and novelty sheds light on their evolutionary importance and biological roles.

Conserved miRNAs

- **Definition**: Conserved miRNAs are defined as those that exhibit substantial sequence similarity among species, demonstrating their evolutionary significance and vital biological roles. Purifying selection is usually used to retain these miRNAs, maintaining their function throughout time [49].

- **Biological Significance**: Certain miRNA families have been shown to be conserved across a wide range of taxa, indicating that these molecules play crucial roles in cellular functions. For instance, research on bilateria, which encompasses a diverse variety of animals, has shown over 30 conserved miRNA families, underscoring their essential regulatory roles in homeostasis and development [50].

- **Expression Patterns**: Studies have demonstrated that conserved miRNAs frequently have more target sites and higher expression levels than novel or species-specific miRNAs. For example, compared to their non-conserved counterparts, conserved miRNAs were found to have more target interactions and the highest average expression levels in cichlid fish [51].

Novel/Species-Specific miRNAs

- **Definition:** miRNAs that are unique to particular organisms or that have just been discovered are referred to as novel or species-specific miRNAs. These miRNAs frequently result from evolutionary responses to particular physiological or environmental settings [52].

- **Evolutionary Adaptations**: Particular characteristics or adaptations that set one species apart from another may be linked to species-specific miRNAs. For instance,
  just nine of the 65 new miRNAs discovered in Brazilian pine (Araucaria angustifolia) were shown to be retained throughout gymnosperms. Most of these were unique to the Araucariaceae family or Brazilian pine, indicating adaptations pertinent to this specific lineage [53].

- **Functional Implications**: Novel miRNAs can nonetheless be very important in controlling developmental processes or reactions to environmental changes, even though their expression may be lower than that of conserved miRNAs. Their distinct patterns of expression can reveal information about the evolutionary stresses that particular species are subject to [54].

MECHANISM OF MICRO RNA

Short, non-coding RNAs known as microRNAs (miRNAs) are essential for the posttranscriptional control of gene expression in a variety of biological settings. They primarily function as negative regulators of gene expression by attaching themselves to target messenger RNAs (mRNAs) and either preventing or encouraging their translation.

Mechanism of miRNA Biogenesis

1. **Transcription and Processing**: RNA polymerase II in the nucleus first transcribes miRNAs as lengthy primary transcripts, or pri-miRNAs. The RNase III enzyme Drosha and its co-factor Pasha (also called DGCR8) 14 make up the Microprocessor complex, which subsequently converts these pri-miRNAs into precursor miRNAs (pre-miRNAs). The pre-miRNA is usually 70 nucleotides long and has a hairpin shape [55].

2. **Export to Cytoplasm**: The pre-miRNA is exported from the nucleus to the cytoplasm by exportin-5 in association with Ran-GTP 4 [56].

3. **Dicer Processing:** After entering the cytoplasm, the pre-miRNA undergoes additional processing by the RNase III enzyme Dicer, which cleaves it into a double-stranded RNA molecule with a length of about 22 nucleotides. One of the two strands in this duplex will grow into miRNA, whereas the other is often broken down [57].

4. **Formation of miRISC**: The mature single-stranded miRNA is then incorporated into the RNA-induced silencing complex (RISC), where it guides the complex to its target mRNAs based on sequence complementarity 12 [58].

Mechanism of Action

1. **Target Recognition**: Although some interactions can take place at the 5' UTRs or within coding areas, miRNA binds to target mRNAs mostly at their 3' untranslated regions (UTRs) [3,4]. This interaction's specificity frequently depends on a "seed" sequence found at the miRNA's 5' end [59].

2. **Gene Regulation**: Upon binding, miRISC can inhibit translation or promote degradation of the target mRNA. This regulation can manifest through:
   - Translational Repression: Inhibition of protein synthesis from mRNA without degrading it [60].
   - mRNA Degradation: Inducing decay of the target mRNA, leading to reduced protein levels [61].

USES OF MICRO RNA

MicroRNAs (miRNAs) have a variety of applications in both diagnostics and therapeutics, as outlined in recent research

1. **Disease Diagnosis Biomarkers**: miRNAs are being used as biomarkers for a number of illnesses, such as cancer and neurological conditions. High specificity and sensitivity non-invasive diagnostic techniques are made possible by their presence in biological fluids such as blood and saliva [62].

2. **Prognostic Indicators**: In illnesses including rheumatoid arthritis and Alzheimer's disease, certain miRNA profiles can help evaluate therapy responses and outcomes by indicating the prognosis and course of the disease [63].

3. **Therapeutic Targets**: miRNAs can be the focus of therapeutic treatments, such as inhibition with antimiRs or replacement therapy with miRNA mimics. Given that miRNAs can act as tumor suppressors or oncomiRs, this strategy holds special promise for the treatment of cancer [64].

4. **Modulators of Drug Resistance**: By altering pathways linked to drug resistance, miRNAs can affect how well therapies work and provide possible techniques to improve therapeutic efficacy [65].

5. **Regulators of Cellular Processes:** They are crucial for controlling cellular processes like metabolism, apoptosis, and differentiation, which makes them crucial for comprehending complicated illnesses [66]

6. **Clinical Trials**: A large number of clinical trials are investigating the potential of miRNAs in a range of medical problems, such as diabetes, heart disease, and many forms of cancer. Validating miRNAs as trustworthy biomarkers or therapeutic agents is the goal of these studies [67].

7. **Personalized Medicine**: Since miRNAs can represent distinct disease states, they are well suited for personalized medicine techniques, which enable customized treatment plans based on distinct miRNA profiles [68].

8. **Applications in Neurodegenerative illnesses**: miRNAs can be used as therapeutic targets and diagnostic tools in neurodegenerative illnesses to reduce neuronal damage and encourage repair processes [69].

9. **Exosomal Functions**: Exosomal miRNAs can influence disease processes and treatment outcomes by regulating immune responses and facilitating cell-to-cell communication [70].

10. **Development of Diagnostic Tools**: A number of diagnostic tools that use miRNA profiling are either in development or currently accessible, such as panels for determining the kinds of cancer or determining the risk of osteoporosis fractures [71].

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