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Review Article

miRNA: A Prospective Tool for Gene Regulation

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Abstract

MicroRNAs (miRNAs) are endogenous small non-coding RNA molecules with an average of ~22 nucleotides in length, found in plants, animals and some viruses. In mammals, miRNA modulates gene expression either by translational repression or mRNA degradation. This is an essential regulatory mechanism to modulate fundamental cellular functions such as differentiation, proliferation, death, metabolism, and pathophysiology of many diseases. In this review, we explore the involvement of several elements in miRNAs pathways as well as the current understanding of both canonical and noncanonical miRNA biogenesis pathways in mammals. We also highlight the available computational tools and algorithms for miRNA target prediction. Finally, we emphasis the various miRNA therapeutic strategies.

Keywords: *miRNA; miRNA Biogenesis; miRNA Therapeutics*

Introduction

In the previous decade, few fields of biology have been transformed as RNA molecular biology. One of the most important discoveries has been the identification of small non-coding RNAs that regulate genes and genomes. This regulation can occur at a different levels of genome function, including chromatin structure, chromosomal segregation, transcription, RNA processing, RNA stability and RNA translation [1]. Based on various aspects of their origins, structures associated with effector proteins and biological activities, three major categories have been recognized: microRNAs (miRNAs), short interfering RNAs (siRNAs) and piwi-interacting RNAs (piRNAs) [2].

The miRNA is a small non-coding RNA molecule with an average of ~22 nt long, found in plants, animals and some viruses. miRNAs are now recognized to have a role in RNA silencing and post-transcriptional regulation of gene expression [3]. There are many different types of cells present in our bodies, such as skin cells, muscle cells, and so on, and each cell possesses identical copies of DNA. The question arise is, if all cells have the same genetic information, why there are so many different types of cells is present? The answer is gene silencing. An explanation of the transmission of genetic information inside a biological system is the central dogma of molecular biology. It is frequently stated that "DNA transcribe into mRNA and mRNA translated into protein". Endogenous miRNA binds to mRNA and prevents the translation of genetic information. If miRNAs are correctly base-pair with mRNAs in a sequence-dependent manner, they inhibit gene expression by degrading target mRNAs and/or preventing their translation through incomplete base pairing with mRNAs. Some miRNAs are found throughout the body, while others

are only found in specific tissues or cell types. This discovery added a new dimension to the understanding of complicated gene regulation networks in both humans and animals.

Embryonic development is a well-organized and highly regulated event. The identification of anatomical and molecular changes helps to classify a developmental stage. Different stages of C. elegans from fertilized egg to embryo, to larva, to adult are easily observed by simple visual inspection. The life cycle of C. elegans is completed in two days and the larva stage is divided into four stages known as L1, L2, L3 and L4 [4]. In these stages, cell division and differentiation are regulated by "heterochronic genes" and mutations in these genes result in the induction of cell fate transformations, such as recapitulation of an earlier phenotype at late stages or early adoption of late-stage phenotypes. The first miRNA, lin-4, was identified in *C. elegans* that does not encode a protein but instead generates a pair of short RNA transcripts that regulate the larval development timing by suppressing the translational of lin-14, which encodes a nuclear protein [5]. They speculated that this regulation was caused by sequence complementarity between lin-4 and unique repeats within the 3′ untranslated regions (UTR) of the lin-14 mRNA. At the end of the first larval stage, lin-14 down-regulation promotes developmental transition into the second larval stage [6]. The second known miRNA, let-7, was expressed later in development and was complementary to the 3′ UTR of the heterochronic genes lin-14, lin-28, lin-41, lin-42, and daf-12, indicating that let-7 may affect the expression of these genes directly. Lin-4 and let-7 are nonhomologous and act similarly to initiate the transition to late-larval and adult stages [7]. Hundreds of miRNAs are now recognized in different organisms and the RNA structure and regulatory mechanisms that have been described in lin-4 and let-7 still provide distinct molecular markers as to what defines miRNAs.

miRNAs are notoriously difficult to differentiate from other types of small RNAs found in the cell, particularly endogenous small interfering RNAs (siRNAs). The most significant distinction between miRNAs and siRNAs is whether or not they suppress their own expression. Almost all siRNAs silence the same locus from which they were produced. On the other hand, most miRNAs silence other genes rather than their own gene [8]. miRNA regulates many aspects of development biology and physiology, therefore understanding their biological role is becoming increasingly important. miRNA expression analysis may provide useful information because dysregulation of its activity can lead to several dysfunctions.

Elements of miRNA pathways

Drosha is a protein with domain architecture belongs to the RNaseIII endonuclease family and is mostly located in the nucleus. Drosha contains proline-rich (P-rich) and arginine/serine-rich (R/S-rich) domains at the N-terminus, followed by a central domain (CED), a tandem of RNase III domains (RIIIDa and RIIIDb) and a double-stranded RNA-binding domain (dsRBD) at the C-terminus (Figure 1(A)) [9]. The N-terminus contains the nuclear localization signal as well as two phosphorylation sites required for Drosha to be targeted to the nucleus. The CED is composed of a platform and a Piwi/Argonaute/Zwille (PAZ)-like a domain that aids in the identification of pri-miRNA [10]. The RIIIDs dimerize intramolecularly to form a composite processing center capable of cutting the 3′ and 5′ strands of the pri-miRNA stem, resulting in staggered ends with 2-nt 3′-overhangs. The dsRBDs of Drosha have weak RNA binding capacity, which is enhanced by DGCR8 [11].

DGCR8 is abbreviated as DiGeorge syndrome Chromosomal Region 8. The N-terminus of DGCR8 contains a nuclear localization signal, followed by the central RNA-binding heme domain (Rhed), two dsRBDs, and the C-terminal tail region (CTT) (Figure 1(B)). The Rhed domain contains a WW motif which is crucial for homodimerization and heme binding via two cysteine residues. It is unclear whether heme plays a role in DGCR8 dimerization [12]. The CTT region is necessary for Drosha binding [13].

DROSHA and DGCR8 together form a Microprocessor complex [14]. The stem structure of pri-miRNA has an apical portion that is terminated by a flexible loop (≥ 10 nucleotides) and flanked by single-stranded segments (≥ 9 nucleotides long) at its basal stem. The miRNA duplex (~22 nucleotides) is imperfectly base-paired, containing G:U wobble pairs whereas the basal stem (~11 nucleotides) is mainly perfectly base-paired, especially at its extremities forming stable platforms (Figure 1(C)) [15]. Microprocessor cleaves at a distance of ~11-bp from the basal junction and ~22-bp from the apical junction. Recent research has identified four primary sequence determinants in the pri-miRNA scaffold (UGUG element in apical loop, GHG (H = A, U, or C) elements in the stem, and UG and CNNC elements in the basal ssRNA overhangs) that con-

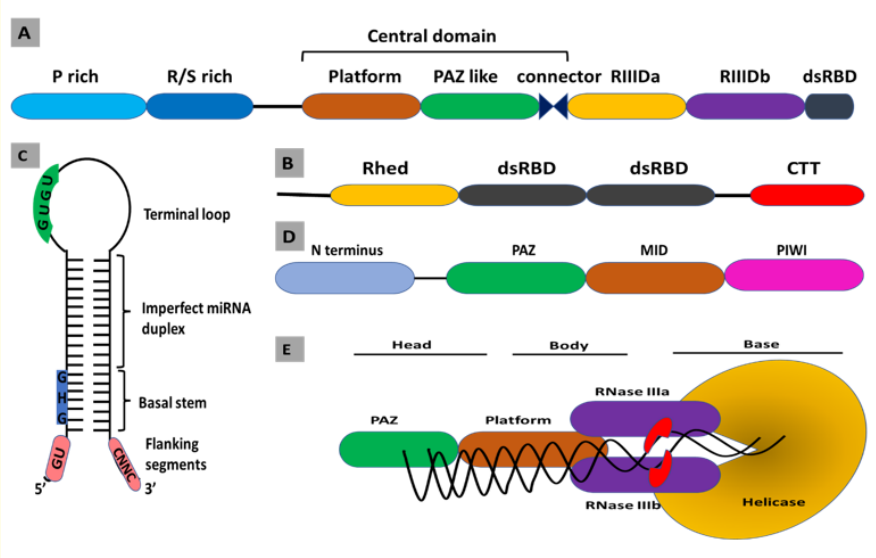

Figure 1: Elements of miRNA pathway (A) Schematic representation of domain architecture of DROSHA (B) Schematic representation of domain architecture of DGCR8 (C) A schematic representation of pri-miRNA sequence. The key invariant nucleotides are highlighted. (D) Schematic representation of domain architecture of ARGONAUTE protein (E) Schematic representation of domain architecture of DICER.

tribute to cleavage specificity and efficiency, thereby imposing additional constraints on Microprocessor recognition [16].

The Dicer enzyme belongs to the RNase III family [17]. The PAZ domain is found at the Dicer's head and contains binding pockets for a dsRNA substrate's 3' overhang [18]. The RNase IIIa and IIIb domains are found on the bottom half of Dicer body's and each domain is thought to be responsible for the cleavage of one strand of the dsRNA substrate [19]. The helicase domain is found at the base of the dicer and forms a clamp near the RNase III domain and stimulates the cleavage of pre-miRNA results into small fragments of around 25 nt in length (Figure 1(E)).

Argonaute (AGO) proteins are small-RNA-binding proteins with a high level of specificity. AGO proteins are directed by small RNAs to complementary target mRNAs, where AGO proteins collaborate with protein binding partners to inhibit translation or induce deadenylation of target mRNAs. AGO proteins have four domains: N-terminal, PAZ, middle (MID) domain and PIWI (P-element induced wimpy testis) (Figure 1(D)). The PAZ domain is responsible for holding the 3'-end of the miRNA and the MID domain having the specific binding pocket for the 5' phosphate of the miRNA. The PIWI domain's structure is similar to that of bacterial RNAse H, which has been demonstrated to break the RNA strand of an RNA-DNA hybrid [20]. Several Ago proteins (e.g., Ago2 in flies and mammals and PIWI subfamily proteins) cleave their target RNAs through their endoribonuclease activity, known as "slicer" to silence their expression [21].

The initial stage in the creation of miRNA RISC (RNA Induced Silencing Complex) is duplex loading, which is a dynamic process that requires ATP hydrolysis. The resulting complex is known as "pre-RISC" and it contains AGO and a short RNA duplex. miRNA RISC maturation can be subdivided into two steps: wedging and passenger expulsion. The N domain of AGO opens one end of the short RNA duplex during wedging step and then two short RNA strands are separated and the passenger strand is discarded from AGO during the passenger ejection step. The complex including AGO and the single-stranded guide is simply referred to as "RISC" [22]. Importantly, the polarity of duplex loading into AGO proteins determines the fate of the two strands—which strand will become the guide or passenger [22]. The relative thermodynamic stabili-

ties of the two ends of the duplex are a significant factor of strand selection; in general, the strand having thermodynamically less stable base pairing at its 5′ end acts as the guide strand.

Biogenesis of miRNA pathway

The miRNAs are encoded in introns or exons of host protein-coding genes, where they can co-transcribe and co-regulated the host gene [23]. The intergenic region encodes the majority of miRNAs (70%). The transcription is controlled by a promoter which is regulated by specific interactions with transcription factors [24]. The biogenesis of miRNA is classified into canonical and noncanonical pathways (Figure 2).

The canonical pathway of miRNA biogenesis

The canonical biogenesis pathway is the predominant pathway for miRNAs processing. Some miRNAs are encoded in clusters, such as the miRNA-17-92 polycistron, whereas others are encoded in host protein-coding genes, where they can be individually transcriptionally regulated by RNA Pol II and then processed into pre-miRNAs by the microprocessor complex, which consists of DGCR8 and Drosha [14]. The Microprocessor is responsible for cleaving the DGCR8-recognized pri-miRNA at distance from the hairpin's base [25]. As a result, a 2 nt 3′ overhang forms on the 70 nt pre-miRNA [26].

Once pre-miRNAs are generated, they are exported to the cytoplasm by an Exportin 5 (XPO5)/RanGTP complex [14,27]. Only pre-miRNAs with adequate stem-loop length and 3'-overhangs are recognized by EXPORTIN-5 and exported from the nucleus for cytoplasmic processing, where they are processed further by the RNase III endonuclease Dicer [28]. The terminal loop is removed during this step, resulting in a ~22 nt mature miRNA duplex [19]. The mature miRNA and its opposing arm are liberated from the pre-miRNA hairpin by DICER, resulting in an imperfectly complementary miRNA:miRNA* duplex [29]. Both strands of the mature miRNA duplex loaded into the Argonaute (AGO) proteins in an ATP-dependent manner [30]. In general, the strand with lowest 5′ stability or 5′ uracil is preferentially loaded into AGO and serves as the guide strand. The unloaded strand is referred to as the passenger strand and it will be unwound from the guide strand through a variety of processes depending on the degree of complementarity. Passenger strands of miRNA with no mismatches are cleaved by AGO2 and degraded by cellular machinery which can produce a strong strand bias. Otherwise, miRNA duplexes with mismatches or non-AGO2 miRNA loaded duplex are passively unwound and degraded [31].

The non-canonical pathway of miRNA biogenesis

Several non-canonical miRNA biogenesis pathways have been described so far. In general, the non-canonical miRNA biogenesis pathway is classified into Drosha/DGCR8-independent and Dicer-independent pathways. In the Dicer-independent pathway, Short hairpin RNA (shRNA) is processed by the microprocessor complex and exported from the nucleus to the cytoplasm via exportin5. In Drosha/DGCR8-independent pathway, mirtrons (which are produced from the mRNA introns) are processed by the splicosome and 7-methylguanosine (m7G)-capped pre-miRNA is exported from the nucleus to the cytoplasm via exportin5 and exportin1, respectively. [32]. These pre-miRNAs require AGO2 to complete their maturation because they are of inadequate in length so they turn and load into AGO2 [33].

All pathways eventually lead to the formation of a mature miRISC complex, which binds to target mRNAs and inhibits translation by interfering with the eukaryotic translation initiation factor (eIF4F complex). The poly(A)-deadenylases PAN2/3 and CCR4-NOT are then recruited by GW182 family proteins linked to Argonaute. Deadenylation is initiated by PAN2/3 and finished by the CCR4-NOT complex, resulting in the removal of the m7G cap on target mRNA, which is subsequently decapped mRNA degraded by the exoribonuclease XRN1 [34].

Function of miRNA pathway

The biological function of miRNAs is highly similar to the RNA interference (RNAi) mechanism. Our understanding of RNAi was that antisense RNA injection suppress protein expression in plants and *C. elegans*. However, in 1998 Fire., *et al.* obtained more efficient RNA silencing in *C. elegans* by injecting dsRNA, whereas ssRNA injection resulted in just a moderate inhibitory impact [35]. This finding implies that RNAi work via a catalytic mechanism rather than by simple antisense binding [35]. As a result, scientists separated the RISC complex and loaded with a miRNA, which is responsible for mediating the miRNA's sequence-specific repressive effects.

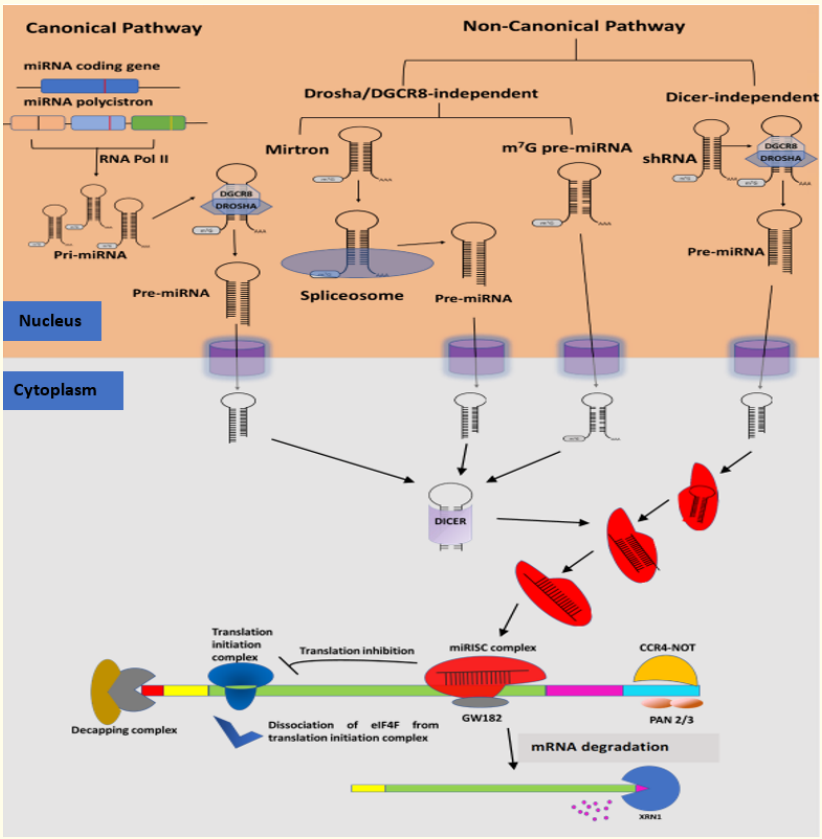

Figure 2: The canonical and non-canonical pathway of miRNA biogenesis.

Most research to far have shown that miRNAs attach to a specific area in the 3′ UTR of their target mRNAs to elicit translational repression [36], while some studies reveal that miRNA also binds to the 5′ UTR, coding sequence and promoter regions [37]. miRNAs that bind to the 5′ UTR and coding areas have been shown to silence gene expression [38], whereas miRNA interactions with the promoter region have been shown to increase transcription [39]. Depending on the degree of complementarity between them, a miRNA regulates the expression of its target mRNA in three ways. A perfect or almost perfect complementarity results in cleavage of target mRNA and inhibition of cap-dependent translation initiation, whereas an imperfect complementarity results in translational repression. Animal miRNAs are often imperfectly complementary to their target sites; so translational repression is the dominant mechanism by which mRNA expression is inhibited in animal cells [5] while in plant miRNAs have perfect or nearly perfect complementarity to their target sites so miRNAs degrade the target mRNAs [40]. The third manner by which miRNA regulates mRNA expression by mRNA deadenylation. When miRNA attaches to the imperfectly complementary target site of mRNA, it accelerates the deadenylation of the poly(A) tail [41].

Computational tools and algorithms of miRNA

Computational methods for miRNA target prediction usually form the backbone of most experimental or in silico miRNA-related pipelines.

Three critical features required for accurate miRNA target prediction [42]:

- The miRNA's 5' seed is complementary to the target mRNA's 3' UTR.
- miRNA target prediction requires only a 7–8 nt seed sequence.
- miRNA-mRNA binding sites are highly conserved from species to species, particularly within the same kingdom.

Computational approaches are the most feasible and effective tools for identifying unknown miRNA–gene interactions and selecting potential candidates for research (Table 1). The sensitivity and precision required for miRNA target prediction can be as high as 60% and 30%, respectively. In miRNA target identification, sensitivity is defined as the percentage of correctly predicted targets out of total (validated) targets in the test dataset (correctly identified targets/all validated targets in the dataset), whereas precision is defined as the percentage of correctly predicted targets out of all predicted targets (correctly identified targets/all predicted targets). TargetScan and miRNA algorithmic network database (miRanda) have been trained to recognize targets in the 3′-UTR region of protein-coding transcripts because miRNA Recognition Elements (MREs) are typically located in these regions. However, miRNA targets in CDS regions have been identified, therefore a microT algorithm has been trained to recognize both the 3′-UTR and CDS regions [43]. Even the best available algorithms fail to detect a significant number of miRNA–gene interactions [44]. So, computational methods derived results cannot be directly utilized, they must be required to be experimentally confirmed.

Application Name	Type
TargetScan	miRNA target prediction
DIANA-microT-CDS	miRNA target prediction
microRNA.org	miRNA target prediction
PicTar	miRNA target prediction
DIANA-TarBase	Manually curated validated miRNA target database
miRTarBase	Manually curated validated miRNA target database
miRecords	Manually curated validated miRNA target database
DIANA-miRPath	miRNA pathway analysis
miRanalyzer	RNA-Seq analysis
miRBase	miRNA sequence and annotation archive

Table 1: Computational tools and algorithms for miRNA target prediction.

miRbase is the key hub and resource for miRNA-related research. miRBase is the primary public repository and online resource for microRNA sequences and annotation. Since then, miRBase has been responsible for microRNA gene nomenclature, providing names for new miRNA gene discoveries. The miRBase website provides sequences, biogenesis precursors, genomic coordinates and context, literature references, deep sequencing expression data, and community-driven annotations for published microRNAs [45]. The miRBase database's most recent version (v22.1) has 38,589 entries representing hairpin precursor microRNAs from 271 organisms. The human genome contains 1917 annotated hairpin precursors and 2654 mature sequences. Drosophila melanogaster has 258 hairpins and 469 mature sequences; *C. elegans* has 253 hairpins and 437 mature sequences and *Arabidopsis thaliana* has 326 hairpins and 428 mature sequences [46].

miRNA therapeutics

The rapidly expanding knowledge on miRNA expression and function is suitable for developing as the next generation of disease therapeutics. The miRNAs are engaged in a variety of clinical conditions and the fact that they induce potent and precise gene silencing makes them attractive therapeutic targets. miRNA's pleiotropic nature makes them particularly attractive therapeutic targets for diseases with a multifactorial origin, however, there are presently no effective therapies approved by FDA [47]. miRNA therapies are less immunogenic than proteins and may be easily chemically modified [48].

miRNA therapies can be approached in two ways. The first method is miRNA antagonists, in which oncogenic miRNAs can be deactivated or silenced by RNA interference-type strategies such as miRNA-specific knockdown by anti-miRNA oligonucleotides (AMOs), miRNA sponges, and miRNA masking [49]. The miRNA antagonists are designed to complementary of targeted miRNA, so miRNA antagonists attach to the targeted miRNA, resulting in the absence of the miRNA-mRNA silencing complex and the loss of miRNA function. The second method is miRNA mimics, in which miRNA with tumor-suppressive abilities are triggered by miRNA restoration strategies, especially miRNA mimics designed to miRNA that mimics to targeted miRNA so miRNA – mRNA silencing complex occur and gain of miRNA (Figure 3) [50].

miRNA antagonists therapy

Anti-miRNA oligonucleotides (AMOs) are single-stranded, chemically modified antisense oligonucleotides (ASOs) ranging in length from 17 to 22 nt and designed to complement a specific miRNA [51]. They function as competitive miRNA inhibitors by annealing to the mature miRNAs and blocking their interaction with target mRNAs. Thus, targeted inhibition of a specific miRNA and subsequent upregulation of its target mRNAs is achievable; however, AMOs have several inherent weaknesses, including their transitory duration of action and inability to target more than one miRNA at a same time. So AMOs are modified to become Locked Nucleic Acid (LNA) anti-miRNAs, which have an extra methylene bridge connecting the 2′-O atom and the 4′-C atom that 'locks' the ribose ring in a C3′-endo or C2′-endo conformation [52]. LNA-modified oligonucleotides have enhanced thermal stability and affinity of Watson-Crick hybridization with their RNA target molecules resulting in improved mismatch discrimination. For example, when antagomir-122 was administered intravenously to mice it remained in their bodies for up to 23 days and suppression of the liver-specific miRNA-122 resulted in reduced levels of plasma cholesterol, which in agreement with the function of the targeted mRNAs [53].

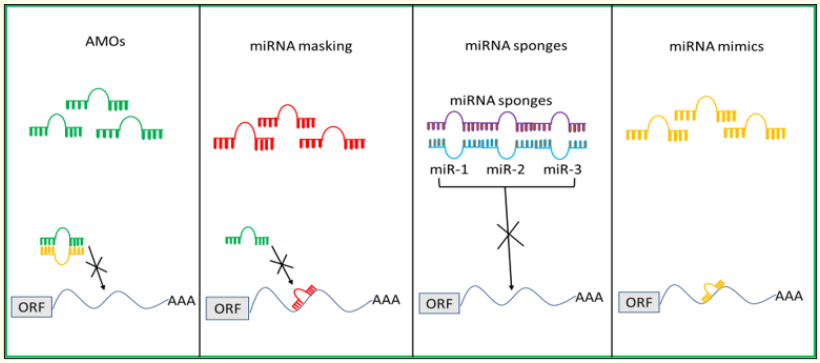

Figure 3: miRNA therapeutics strategies.

mRNA has several tandem binding sites for an interested miRNA, causing 'miRNA sponges' to compete with native miRNA targets for miRNA binding to mRNA. This sponge effect prevents miRNAs for doing their function, resulting in enhanced mRNA expression [54]. For example, inhibition of miRNA 9, which is upregulated in breast cancer cells and directly targets CDH1, using a 'miRNA sponge' by attaching to the miRNA 9 and inhibits the metastasis formation [55].

miRNA masking is a miRNA knockdown strategy that differs from the AMO approach in that it targets mRNAs in a gene-specific manner. A miRNA-mask is a single-stranded 2′-O-methylmodified oligo ribonucleotide that possesses perfect complementarity to an endogenous miRNA binding site in the 3′ UTR of a protein-coding mRNA gene. As a result, miRNA-mask attaches to the targeted mRNA and inhibits miRNA from binding to the targeted mRNA, therefore inhibiting miRNA's repressive effect. For example, miRNA-mask inhibited the repressive effect of miRNA-430 on transforming growth factor beta (TGFß) by using a miRNA-mask that was complementary to the miRNA-430 binding site in its target mRNAs squint (sqt) and lft2 [56].

miRNA mimics therapy

The basic idea behind miRNA mimics therapeutic techniques for various diseases is to restore the expression level of those miRNAs which suppress the disease states. miRNA mimics are synthetic double-stranded oligonucleotides that are converted to single-stranded miRNA-like molecules that function similarly to endogenous miRNAs. For example, miRNA-26a is a suppresser of hepatocellular carcinoma cancer (HCC). The restoration of miRNA-26a in the therapy of HCC in mice resulted in cancer growth suppression and the initiation of apoptosis [57].

An increased number of reports suggest that miRNAs have substantial usefulness as biomarkers for pathogenic diseases,

modulators of medication resistance, and/or medicines for medical intervention in almost all human health issues. Trials have been conducted or are actively recruiting patients to investigate the profiles of non-coding RNA transcripts in a variety of disease conditions including coronary heart disease, breast cancer, diabetes, depressive disorder, stroke, lupus, epilepsy, addison's disease, influenza, liver disorder and even toxic exposure to agents such as acetaminophen. Furthermore, miRNAs are present throughout the body and may be easily quantified in peripheral blood, saliva, urine, tissue biopsies, cerebrospinal fluid (CSF) and other biological samples [58]. miRNAs also cross the blood-brain barrier, so blood, plasma and serum testing indicate many neurodevelopmental and neurodegenerative disorders. Indeed, the biotech company DiamiR's focus mostly on miRNA diagnostic blood tests to assess brain activity [47].

The FDA just authorized first small-interfering RNA (siRNA) drug, making this an exciting time for therapeutic short RNA (less than 200 nucleotides in length). Patisiran, a siRNA drug, has been approved for the treatment of a rare polyneuropathy caused by hereditary transthyretin-mediated (hATTR) amyloidosis. It acts by degrading the mRNA transcript for transthyretin [59]. Despite the fact that miRNA treatments have not yet been approved by the FDA for phase 1 and phase 2 clinical trials. Academic institutions, biotech companies and the pharmaceutical industry are all involved in the clinical research efforts. There are three biotech companies primarily focused on expanding miRNA-related therapeutics, such as Miragen, MiRNA Therapeutics (now Synlogic) and Regulus Therapeutics [47].

In 2016, the biotech company Synlogic halted phase 1 studies of their miRNA-34 medication mimic, MRX34, for cancer therapy after five patients developed strong immune reactions. Future phase 2 studies of MRX34 for melanoma were also halted due to the appearance of substantial immune reactions. The miRagen company also announced a phase 1 trial using MRG 110 with potential therapeutic applications in wound healing and heart failure. Miragen is also conducting a phase 1 trial for miR-29 (MRG-201) to treat keloid and scar tissue formation, as well as a phase 2 trial for miR-155 (Cobomarsen; MRG-106) in T-cell lymphoma patients. In 2019, Regulus company announced their new miRNA drug candidate called RGLS5579 which targets miR-10b for prospective trials in patients with glioblastoma multiforme, one of the most severe kinds of brain cancer with a median survival of around 14.6 months [60].

The newer technology, "TargomiR", demonstrated promising results in individuals with recurrent malignant pleural mesothelioma or non-small cell lung cancer. TargomiR is a delivery vehicle that consists of a miRNA mimic, bacterial generated minicells and a targeting moiety (i.e., a specific antibody that recognizes a protein on target cells). The miRNA mimic in the first human trial of a TargomiR medication, MesomiR-1, was the previously discovered tumor-suppressing transcript miRNA-16, and the targeting moiety was an antibody to the epidermal growth factor receptor (EGFR), which is persistently deregulated in lung cancer cells [61]. These findings provide new hope to mesothelioma patients in which less than 10% survive more than 5 years [62]. Overall, this research and publication suggest that miRNA medications have a promising future in disease therapeutics.

Conclusions

The small non-coding miRNA undoubtedly one of the most significant discoveries in the field of RNA molecular biology. However, the lack of knowledge regarding miRNA target genes delays a complete understanding of miRNA biological activities. Computational tools are the most feasible and effective approaches for detecting undiscovered miRNA–gene interactions and selecting promising candidates for experiments. As a result, further research is needed to identify miRNA target genes for either downregulated or upregulated miRNAs. There are several clinical research and prognostic therapies are currently underway to establish the effectiveness of microRNA-based therapeutics against a wide range of fatal diseases. Because of the increasing interest in microRNAs, numerous small and long non-coding RNAs have been found for effective therapeutic applications in the short and long-term treatment of a range of diseases. So, this is an evolutionary method for treating various types of diseases.

Conflict of Interest

Authors have no conflict of interest.

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