International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

MicroRNA processing, role of GW182 Scaffolding proteins in RNA interference and Potential roles of let-7, miR-15 and miR-29 families as tumor suppressors

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International Journal of Science and Research Archive, 2023, 08(01), 357–366

Publication history: Received on 04 December 2022; revised on 15 January 2023; accepted on 18 January 2023

Article DOI: https://doi.org/10.30574/ijsra.2023.8.1.0046

Abstract

MicroRNAs are class of non-coding RNAs which are involved in many fundamental cellular and physiological process such as cell differentiation, proliferation and regulation of cell cycle and apoptosis. The vast majority of microRNAs are transcribed from non-coding regions (introns) of genes into primary miRNAs, which are then processed into precursor miRNAs and, lastly, microRNAs. Humans have at least 277 genes that are making microRNAs and these are conserved in mammals. MicroRNAs control gene expression by the sequence-specific targeting of mRNAs, which results in translational repression or mRNA destruction. Recent research has revealed that miRNAs have a role in tumor development, progression, and metastasis. MiRNA function dysregulation is linked to a growing variety of human disorders, including cancer, with miRNAs serving as tumor suppressors or oncogenes (oncomiRs). This review attempts to offer a snapshot of aspects of miRNA biogenesis, processing, role of GW182 scaffold proteins in microRNA mediated gene silencing and potential role of some microRNA families as tumor suppressors.

Keywords: MiRNA; Biogenesis; Gene silencing; DICER; RISC; Tumor suppressors; Argonaute proteins; GW182

1. Introduction

In 1993 Lee and coworkers discovered the first microRNA, LIN-14, in the nematode Caenorhabditis elegans. The downregulation of the LIN-14 protein was discovered to be important for the passage of these organisms from the first larval stage (L1) to L2. Furthermore, it was shown that the downregulation of LIN-14 is reliant on the transcription of a second gene known as lin-4. Surprisingly, the transcribed lin-4 did not result in the production of a physiologically active protein. Instead, it produced two short RNAs with lengths of 21 and 61 nucleotides, respectively. The longer sequence was a stem-loop structure that acted as a precursor for the shorter RNA. Later, this group discovered, in collaboration with Wightman, that the smaller RNA had antisense complementarity to many locations in the 3′ UTR of lin-14 mRNA. The binding of these complementary areas reduced LIN-14 protein expression without having any noticeable effect (1).

MicroRNAs (miRNAs) are short, non-coding RNAs that influence gene expression. They have an average length of 19-25 nucleotides. MiRNAs are thought to control around 30% of human genes (2). Most miRNAs are transcribed from DNA sequences into primary miRNAs (pri-miRNAs) by RNA polymerase-2 and processed into precursor miRNAs (pre-miRNAs) and mature miRNAs. In most cases, miRNAs interact with the 3′ UTR of target mRNAs to suppress expression (3). However, interaction of miRNAs with other regions, including the 5′ UTR, coding sequence, and gene promoters, have also been reported (3). More than half of the miRNA's genes are located in cancer-associated genomic regions or in fragile sites. Specific miRNA signatures have been associated with distinct subsets of solid tumors and hematological malignancies miRNAs can act as tumor suppressors when their function loss can initiate or contribute to the malignant transformation of a normal cell. The loss of function of a miRNA could be due to several mechanisms, including genomic

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deletion, mutation, epigenetic silencing, and/or miRNA processing alterations (4). In this review we briefly describe miRNA biogenesis, processing, and how they regulate gene expression. We have also described the potential roles of some microRNA families on how they act as tumor suppressors.

2. MiRNA Biogenesis and Processing

MiRNA biogenesis starts with the processing of RNA polymerase II/III transcripts post- or co-transcriptionally (3). About half of all currently identified miRNAs are intragenic and processed mostly from introns and relatively few exons of protein coding genes, while the remaining are intergenic, transcribed independently of a host gene and regulated by their own promoters.

The most frequent and prevalent mechanism for processing miRNAs is through the canonical pathway, which is where microRNAs are produced. In this pathway, the microprocessor complex, which is made up of the ribonuclease III enzyme Drosha and the RNA binding protein DiGeorge Syndrome Critical Region 8 (DGCR8), converts pri-miRNAs from their genes into pre-miRNAs (5). Within the pri-miRNA, DGCR8 detects an N6-methyladenylated GGAC as well as other motifs (6), while Drosha cleaves the pri-miRNA duplex at the base of the characteristic hairpin structure of pri-miRNA. This results in the formation of a 2 nt 3′ overhang on pre-miRNA (7). Once pre-miRNAs are generated, they are exported to the cytoplasm by an exportin 5 (XPO5)/RanGTP complex and then processed by the RNase III endonuclease Dicer (8).

This processing involves the removal of the terminal loop, resulting in a mature miRNA duplex [figure-1]. The nomenclature of the mature miRNA form is determined by the directionality of the miRNA strand. The 5p strand grows from the 5′ end of the pre-miRNA hairpin, whereas the 3p strand grows from the 3′ end (9). Both strands of the mature miRNA duplex may be loaded into the Argonaute (AGO) family of proteins (AGO1-4 in humans) in an ATP-dependent way (10). For any given miRNA, the proportion of AGO-loaded 5p or 3p strand varies greatly depending on the cell type or cellular environment, ranging from near equal proportions to predominantly one or the other (11). The selection of the 5p or 3p strand is based in part on the thermodynamic stability at the 5′ ends of the miRNA duplex or a 5′ U at nucleotide position 1 (12).

Generally, the strand with lower 5′ stability or 5′ uracil is preferentially loaded into AGO, and is deemed the guide strand. The unloaded strand is called the passenger strand, which will be unwound from the guide strand through various mechanisms based on the degree of complementarity (12). The passenger strands of miRNA that contain no mismatches are cleaved by AGO2 and degraded by cellular machinery which can produce a strong strand bias. Otherwise, miRNA duplexes with Deep sequencing methods have identified multiple types of RNA molecules that mimic miRNAs structurally and functionally (10).

However, they do skip one or more stages in the normal biogenesis process. As a result, these miRNAs are known as noncanonical miRNAs. It is important to note that Dicer is essentially always required for the generation of both canonical and non-canonical miRNAs, and that without it, nearly all functional miRNAs are lost (13). Drosha and Dgcr8 are solely required for canonical miRNA processing, while non-canonical miRNAs can be generated in their absence. In other words, the deletion of Drosha or Dgcr8 causes the complete loss of canonical miRNA, but preserves non-canonical miRNA biogenesis (13).

The mirtron pathway was the first non-canonical pathway found. This parallel pathway uses Dicer in the cytoplasm but does not require the Drosha/Dgcr8 complex in the nucleus to create pre-miRNAs. Mirtrons were identified by analyzing deep sequencing data from tiny RNAs from Drosophila melanogaster (14) and Caenorhabditis elegans (15), which showed pre-miRNA-sized short introns. These introns are processed in the nucleus by spliceosomes and debranching enzymes to form miRNA hairpins that are directly appropriate for Dicer cleavage. This hairpin is then exported to the cytoplasm by Exportin-5 and cleaved by Dicer (13). Thus, the mirtron pathway bypasses the microprocessor processing, or rather replaces it with splicing activity, and then merges with the canonical miRNA pathway at the Exportin-5-bound transport stage (13).

Plant microRNA biogenesis varies from animal biogenesis primarily in the nuclear processing and export processes. First, in plants, a single enzyme, DCL1, located within the nucleus, performs the functions of both Drosha and Dicer, converting pri-miRNAs to mature miRNAs. Second, before plant miRNA-miRNA* duplexes are exported from the nucleus, their 3' overhangs are methylated by HEN1, an S-adenosyl methionine-dependent methyltransferase.

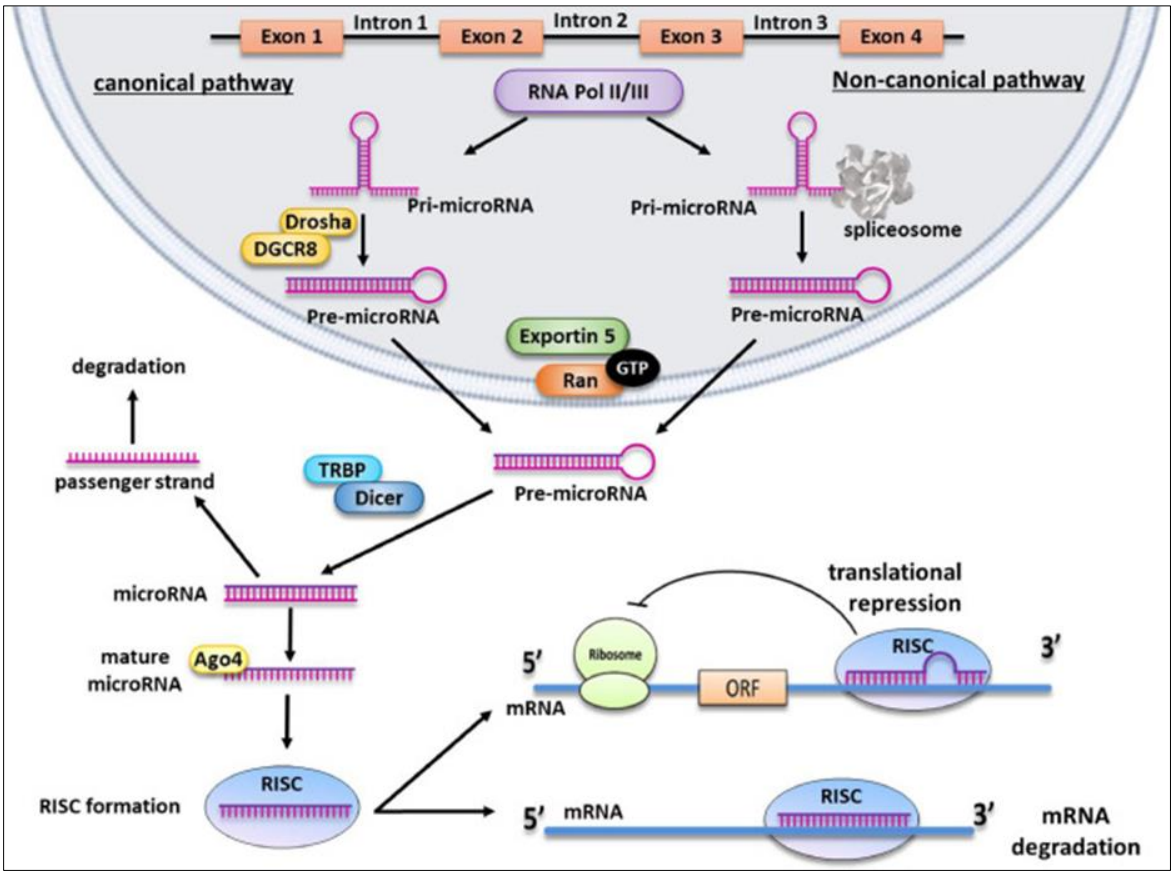

Figure 1 The mechanism of microRNA biogenesis and its regulation of gene expression

3. Understanding the Role of GW182 Scaffolding proteins in RNA interference

GW182 was initially identified as an autoantigen found in cytoplasmic structures and related with mRNA (16) (17). Later, it was shown that GW182 binds argonaute (AGO) protein and plays an important role in translational repression during RNA interference (RNAi) (18) (19) (20). According to studies, GW182 bonded to AGO2 and formed a link between AGO2 and proteins involved in mRNA decapping and degradation (21; 22; 23; 24; 25; 26; 27; 28).

In human cells, there are three GW182 paralogs: trinucleotide repeat containing 6A (TNRC6A), TNRC6B, and TNRC6C. Human GW182 is another name for the TNRC6A paralog. GW182 is a multidomain protein with an N-terminal domain capable of binding one or more copies of AGO2 (29; 30; 31; 32). Many glycine-tryptophan (GW) repeats in the AGO binding domain operate as anchors for protein-protein interactions, and these GW repeats are required for association with AGO2. GW182 also has C-terminal domains that can bind with other proteins and silence bound transcripts independently of AGO (33).

Inside cells, RNAi proteins and short RNAs create a programmable ribonucleoprotein complex that allows for sequence-specific identification of RNA targets and gene expression regulation (34). The sequence-selectivity of recognition is determined by the small RNA, whereas AGO protects the small RNA and facilitates effective search and recognition of target sequences (35). The most well-known examples of RNA-mediated gene expression regulation are translation inhibition by micro RNAs (miRNAs) and small interfering RNAs (siRNAs). MiRNAs often have several cellular targets that are not exactly complementary, whereas siRNAs typically have one target that is absolutely complementary (36).

It is generally understood that siRNAs and miRNAs operate in the cytoplasm. RNA interference factors, such as AGO2 and TNRC6, are found in mammalian cell nuclei (37) and regulate gene transcription and splicing (38; 39). Both transcriptional silencing and transcriptional gene activation can be used to regulate transcription (40; 41).

4. Overview of MicroRNA mediated Gene Silencing

Gene silence can occur by mRNA degradation or by preventing mRNA from being translated. To exert their regulatory function miRNAs, associate with Argonaute (AGO) family proteins to form the basic core of miRNA-induced silencing complexes (miRISCs) (24). If the miRNA and mRNA sequences are completely complementary, Ago can cleave the mRNA, resulting in direct mRNA destruction.

The target specificity of miRISC is due to its interaction with complementary sequences on target mRNA, called miRNA response elements (MREs). The degree of MRE complementarity determines whether there is AGO-dependent slicing of target mRNA or miRISC-mediated translational inhibition and target mRNA decay (25). If perfect complementarity is not present, silence is performed by inhibiting translation. MiRNAs can detect their target mRNAs via the seed sequence present at the 5' end in the situation of incomplete complementarity [Figure-2]. A miRNA's seed sequence is defined as the first 2-8 nucleotides from the 5' end to the 3' end.

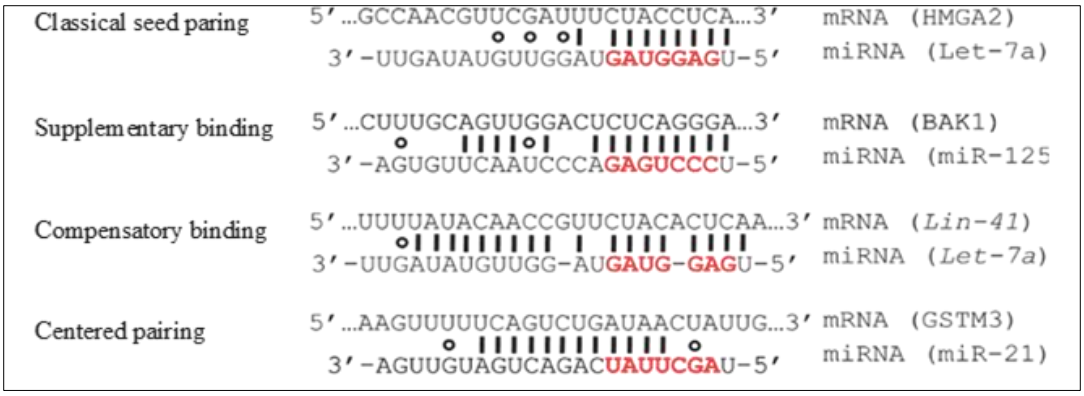

Figure 2 Major possibilities in binding of miRNA-target mRNA. Seed sequence of mature miRNA in bold and red, solid lines indicate perfect Watson-Crick complementary and circles demonstrate G:U wobble. (26)

In animals the mRNA targets are often only partially complementary to the miRNA, which precludes cleavage by AGO protein (27). Moreover, in humans, only AGO2 is catalytically active, whereas AGO1, AGO3 and AGO4 are not (27). Silencing occurs through a combination of translational repression, deadenylation, decapping and 5′-to-3′ mRNA degradation (42).

5. Mechanism of GW182 – miRNA mediated gene silencing

GW182 proteins bounds with AGO family proteins to play an essential role in miRNA-mediated gene silencing, as evidenced by the fact that deletion of these proteins in human, D. melanogaster, and Caenorhabditis elegans cells substantially impairs gene silencing (43; 44; 45; 46; 47; 48; 49; 50).

The GW182 proteins play a central part in this process and are among the most extensively studied AGO partners (51) (52) (53). They function as flexible scaffolds to bridge the interaction between AGO proteins and downstream effector complexes, such as the cytoplasmic deadenylase complexes PAN2–PAN3 (polyadenylate binding protein dependent poly(A) nuclease complex) and CCR4–NOT (carbon catabolite repression 4 (CCR4)–negative on TATA-less (NOT) complex) (42).

Animal microRNAs (miRNAs) linked to the Argonaute (AGO) protein in miRNA-induced silencing complexes (miRISCs) detect their mRNA targets by base-pairing to partially complementary binding sites, which are mostly situated in the mRNA's 3 untranslated region. AGO proteins associate with the GW182 protein, which in turn interacts with the cytoplasmic poly(A)-binding protein (PABPC) and the PAN2-PAN3 and CCR4-NOT cytoplasmic deadenylase complexes (54). The PAN2-PAN3 and CCR4-NOT complexes catalyse the deadenylation of the mRNA target (55). The GW182 proteins contain an AGO-binding domain (ABD) and a silencing domain. Deadenylated mRNAs are decapped and rapidly degraded by 5 to-3' exoribonuclease-1(XRN1) in animal cell cultures. Furthermore, miRNAs inhibit translation, but the specific molecular mechanism underlying this is unknown. The increasing consensus is that miRNAs restrict translation initiation by interfering with the activity and/or assembly of the eukaryotic initiation factor 4F (eIF4F) complex. The eIF4F complex is made up of the cap-binding protein eIF4E, the adaptor protein eIF4G, and the DEAD box

RNA helicase eIF4A (56). EIF4G acts as a scaffold for protein-protein interactions that are required for the recruitment of the 43S pre-initiation complex and translation initiation (57).

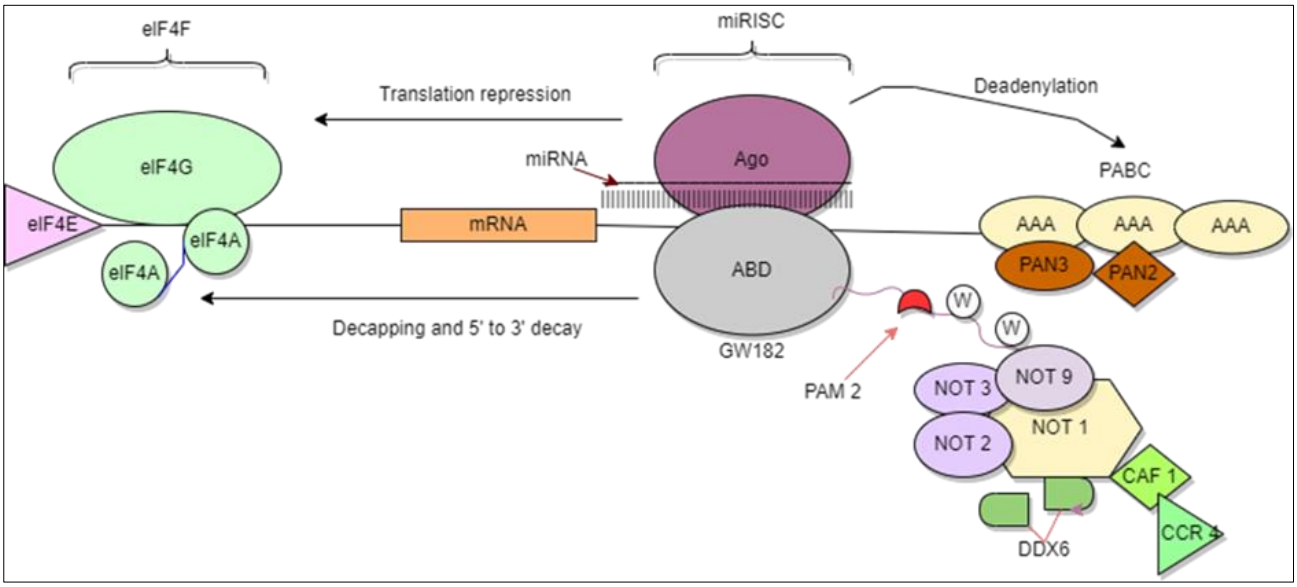

Figure 3 Overview of miRNA-mediated gene silencing in animals

6. Role of let-7 as Tumour suppressor's

MiRNA can act as a tumor suppressor when its function loss can initiate or contribute to the malignant transformation of a normal cell. The loss of function of miRNA could be due to several mechanisms, including genomic deletion, mutation, epigenetic silencing and/or miRNA processing alterations (58; 59; 60; 61).

Let-7 micro-RNAs are first known miRNAs in humans and these are highly conserved (62; 63; 64; 65).

Any dysregulation of these let-7 micro-RNAs could lead to impaired development of cell [cancer].

let-7 miRNAs were found to regulate human RAS [Rat sarcoma] oncogene expression and to be often down-regulated in human lung tumors (66). The 3'UTRs of the human RAS genes contain multiple LCSs [let-7 complementary sites], allowing let-7 to regulate RAS expression.let-7 expression is lower in lung tumors than in normal lung tissue, while RAS protein is significantly higher in lung tumors (67).

HMGA2, a high-mobility group protein, is oncogenic in a variety of tumors, including benign mesenchymal tumors and lung cancers. HMGA2 was derepressed upon inhibition of let-7 in cells with high levels of the miRNA. Ectopic expression of let-7 reduced HMGA2 and cell proliferation in a lung cancer cell. The effect of let-7 on HMGA2 was dependent on multiple target sites in the 3'untranslated region (UTR), and the growth-suppressive effect of let-7 on lung cancer cells was rescued by overexpression of the HMGA2 ORF [open reading frame] without a 3'UTR (68).

Let-7 may function as one of the growth suppressive miRNAs in human colon cancer cells. When let-7 low-expressing DLD-1 human colon cancer cells were transfected with let-7a-1 precursor miRNA, which is located at chromosome 9q22.3, the cells underwent significant growth suppression. At that time, the levels of RAS and c-myc proteins were lowered after the transfection, whereas the levels of both of their mRNAs remained almost unchanged. These findings suggest the involvement of let-7 miRNA in the growth of colon cancer cells (66).

7. Role of miR-15 as tumour suppressors

B-cell chronic lymphocytic leukemia (CLL) is the most common adult leukemia. The most common chromosomal abnormalities detectable by cytogenetics include deletion at 13q (69). Deletions and translocations involving two miRNAs, miR-15a and miR-16-1, located in a cluster at 13q14.3, and their down-regulation was found in 65% of B cell CLL patients (70).

Expression of these miRNAs is also downregulated in, melanoma, colorectal cancer, bladder cancer and other solid tumors. MiR-15/16 cluster targets multiple oncogenes, including BCL2, Cyclin D1, MCL1 and others (71). The most important target of miR-15/16 in CLL is arguably BCL2, as BCL2 is overexpressed in almost all CLLs (72).

A series of luciferase assay tests were performed to demonstrate that miR-15/16 directly targeted BCL2. A short portion of the 3′ UTR of BCL2, anticipated to interact with miR-15/16, was cloned into the 3′ UTR of the luciferase gene (73). When the WT [wild type] construct was employed, miR-15/16 expression dramatically lowered luciferase activity, however no difference was seen when a construct containing a mutant microRNA-mRNA interaction site was used [78]. This demonstrated that both microRNAs interact with and suppress BCL2 expression (73). Overall, our findings suggest that loss of miR-15/16 is the primary driver of BCL2 overexpression in CLL.

Investigations were carried out to know miR-15/16 functions as a tumor suppressor. A group of researchers transiently transfected MEG-01 cells, which do not express miR-15/16, with miR-15/16 or an empty vector and investigated their tumorigenic activity in immunocompromised mice. After 4 weeks, no tumors developed from cells transfected with miR-15/16, but big tumors developed from cells transfected with the empty vector (72). As a result, it was determined that miR-15 and miR-16 act as tumor suppressors in MEG-01 leukemia cells (72).

Surprisingly, miR-15/16 exhibits tumor-suppressor effects in cancers other than lymphoid malignancies (74). The expression of miR-15/16 is downregulated in malignant pleural mesothelioma. Transfection of synthetic oligos imitating miR-15/16 inhibited proliferation in mesothelioma cell lines and decreased tumor sizes in xenograft models (74).

8. Role of miR-29 as tumour suppressors

Members of the miR-29 family have gained interest as tumor suppressors because they are silenced or downregulated in several types of cancer (75; 76).

The human miR-29 family consists of three closely related precursors, with miR-29a and miR-29b1 being transcribed from chromosome 7 (7q32.3), and miR-29b2, which has an identical sequence to miR-29b1, as well as miR-29c being transcribed from chromosome 1 (1q32.2) (77).

Interestingly, deletions on chromosome 7q32 are common in myelodysplasia and therapy-related acute myeloid leukemia (AML) (78). Indeed, members of the miR-29 family have been found to be downregulated in CLL, lung cancer, invasive breast cancer, AML, and cholangiocarcinoma (79; 80). MiR-29b promoted apoptosis in cholangiocarcinoma and lung cancer cell lines and decreased tumorigenicity in a lung cancer xenograft model (81; 82). These potent tumor suppressor effects are explained in part by the miR-29 family's direct targeting of the antiapoptotic protein MCL-1 (myeloid leukemia cell differentiation protein) and the oncogene TCL-1 (T cell leukaemia-1) (83).

Endogenous miR-29 binds directly to the 3'UTR of Mcl-1, inhibiting Mcl-1 protein production and promoting tumor cell death. Furthermore, the miR-29 family members (miR-29a, miR-29b, and miR-29c) raise p53 levels and trigger apoptosis in a p53-dependent manner (84). Researchers discovered that members of the miR-29 family may directly decrease p85a (the regulatory subunit of PI3 kinase) and CDC42 (a Rho family GTPase) (85). p85a and CDC42 negatively regulate p53 and then trigger apoptosis via a p53-dependent signaling pathway. Both miR-29a and miR-29b not only target anti-apoptotic genes, but also upregulate pro-apoptotic genes such BIM (BCL2L11) and the tumor suppressor PDCD4 (86).

Abbreviation

RAS - A family of genes that make proteins involved in cell signaling pathways that control cell growth and cell death. Mutated (changed) forms of the RAS gene may be found in some types of cancer. These changes may cause cancer cells to grow and spread in the body.

9. Conclusion

From the date of discovery miRNAs have evolved as tumor suppressors in variety of cancers. A greater understanding about the mechanisms of miRNA mediated gene silencing led to discovery of many therapeutic agents which enabled the development of therapeutics to fight or prevent many cancers. Further studies are required for optimizing the stability of miRNAs and repression of tumor suppressor miRNAs. Methods and procedures for knowing how miRNAs respond to heterogenicity of tumors and safety of miRNAs in vivo has yet to be evaluated.

Compliance with ethical standards

Acknowledgments

This endeavor would not have been possible without guidance of my fellow researchers and I'm deeply indebted to their work in writing this article.

Disclosure of Conflict of interests

No conflict of interests.

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