Transcriptional regulation of nuclear miRNAs in tumorigenesis (Review)

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Abstract. MicroRNAs (miRNAs/miRs) are a type of endogenous non-coding small RNA that regulates gene expression. miRNAs regulate gene expression at the post-transcriptional level by targeting the 3'-untranslated region (3'UTR) of cytoplasmic messenger RNAs (mRNAs). Recent research has confirmed the presence of mature miRNAs in the nucleus, which bind nascent RNA transcripts, gene promoter or enhancer regions, and regulate gene expression via epigenetic pathways. Some miRNAs have been shown to function as oncogenes or tumor suppressor genes by modulating molecular pathways involved in human cancers. Notably, a novel molecular mechanism underlying the dysregulation of miRNA expression in cancer has recently been discovered, indicating that miRNAs may be involved in tumorigenesis via a nuclear function that influences gene transcription and epigenetic states, elucidating their potential therapeutic implications. The present review article discusses the import of nuclear miRNAs, nucleus-cytoplasm transport mechanisms and the nuclear functions of miRNAs in cancer. In addition, some software tools for predicting miRNA binding sites are also discussed. Nuclear miRNAs supplement miRNA regulatory networks in cancer as a non-canonical aspect of miRNA action. Further research into this aspect may be critical for understanding the role of nuclear miRNAs in the development of human cancers.

Contents

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1. Introduction

MicroRNAs (miRNAs/miRs) are 21-24 nucleotide single-stranded non-coding small RNAs that play a crucial role in the regulation of gene expression (1). miRNAs have been shown to play a key role in the cellular response to environmental stresses, such as starvation, hypoxia, oxidative stress and DNA damage. The biological importance of miRNAs has been highlighted in recent decades by the deregulation of miRNA expression in a variety of human diseases, including cancer (2,3). Several studies have found that the dysregulation of miRNA expression is closely linked to tumor initiation, growth and metastasis (4-6). Numerous miRNAs can function as oncogenes or tumor suppressors, causing cancer or suppressing tumor growth (7,8). Several miRNAs have been found to be involved in epigenetic, transcriptional and post-transcriptional processes (9,10). miRNA dysfunction disrupts the expression of oncogenic or tumor-suppressing target genes, which is implicated in cancer pathogenesis. The majority of mature miRNAs are known to interact with the 3'UTR of target mRNAs via complete or incomplete pairing to induce mRNA degradation or translational inhibition (11,12). miRNAs are loaded onto Argonaute (AGO) proteins in the cytoplasm to direct the RNA-induced silencing complex (RISC), which is composed of AGO, DICER, TAR-RNA-binding protein (TRBP) and trinucleotide repeat containing 6 (TNRC6), to bind to the 3'UTR (13). Post-transcriptional gene silencing in the cytoplasm is a well-known function mediated by miRNAs through the miRNA-induced silencing complex (miRISC). Furthermore, certain studies have suggested that it occurs in the 5'-UTR (14) and even in protein-coding sequences (15). However, interactions of miRNAs with other regions, such as gene promoters, have been reported (16,17). miRNAs are active in the nuclei, according to research, and several miRNAs that are prominently localized in the nucleus are associated with transcriptional regulation. To support this, the majority of miRNAs have recently been reported to be imported back into the nucleus following maturation,

with abundant putative miRNA target sites in gene promoters in both the sense and antisense orientations, which regulate transcriptions in the nucleus by binding to complementary sequences in the promoters (18-20) of target genes. miRNAs have been found to have two functions: Transcriptional gene activation (TGA) and transcriptional gene silencing (TGS) (21). It is now clear that the transcriptional regulation of nuclear miRNAs can promote the occurrence and progression of a variety of malignant tumors, including gastric, prostate cancer (Pca) and lung (22-24). In recent years, there has been a growing body of evidence indicating an epigenetic interaction between DNA methylation modification and nuclear miRNA expression in cancer (24-26). Several mature miRNAs have been presently identified to be enriched in the nuclei of various tissues and cell types using evolving technologies such as high-throughput sequencing (27). These findings shed new light onto the mechanisms and treatment strategies of miRNAs in the control of tumor gene expression (23). However, the types and numbers of miRNAs found in the nuclei of different cells, as well as their potential biological regulatory functions, remain unknown (28). The understanding of the time frame and associated mechanisms through which nuclear miRNAs regulate transcription in tumor cells is particularly limited. As a result, it is of utmost importance to investigate the regulatory networks of gene expression that not only cover nuclear miRNA expression, but also its immediate effects on different tumor cell types for cancer treatment by targeting or manipulating the expression profile of nuclear miRNAs.

The present review article discusses a number of processes involved in the biogenesis of miRNAs and their transport into the nucleus. The structure, function and roles of miRNA in the regulatory effects on AGO proteins are also discussed. In addition, some software tools for predicting miRNA binding sites in order to identify and predict the corresponding miRNAs are noted and summarized. It is demonstrated that nuclear miRNAs, through epigenetic modifications, mediate the regulatory function of TGA or TGS, as well as their importance in tumorigenesis and development. The aim of the present review was to discuss the endogenous function of nuclear miRNAs in cancer cell growth and to propose potential applications in disease treatment, such as the development of drug targets via miRNAs (29).

2. The process of miRNAs entering the nuclei

The majority of miRNAs have their own promoters that exist in the genome on their own (30). The promoter, in conjunction with RNA polymerase II (RNAP II), transcribes the genes encoding miRNAs into primary miRNA transcripts (pri-miRNAs) (31). A microprocessor complex (32) converts these pri-miRNAs into short 70-nucleotide stem-loop structures known as precursor miRNAs (pre-miRNAs) in the nucleus. The DROSHA RNase III enzyme and the Di George syndrome critical region 8 double-stranded-RNA binding protein comprise the microprocessor complex (DGCR8). DROSHA cleaves the primary miRNAs in order to remove a portion of the unpaired RNA sequences (33).

The nuclear export machinery, which consists of Exportin-5 complexed with GTPase (Ran GTP), transports the pre-miRNAs to the cytoplasm, where they are converted into mature miRNAs, a 22-nt miRNA duplex, by cleavage of the hairpins by the enzyme DICER (34). The asymmetry rule is a strand-selection mechanism in which only one strand is preferentially retained to form the functional miRISC, while the other strand is degraded. In most cases, the miRISC induces mRNA decay and translational suppression by interacting with complementary sequences in the 3'UTR of target gene mRNAs (Fig. 1). Several previous studies have found that miRISC is imported into the nucleus of mammalian cells, and that nuclear RISC (an ~158 kDa complex) is smaller than its cytoplasmic counterpart (a 20-fold larger complex of nearly 3 MDa). Moreover, several RISC components have been identified to function in the nucleus (21). Western blot analysis has revealed the presence of AGO protein, DICER, TRBP and GW182/TNRC6 in the nucleus, where they combine to form multi-protein complexes (35). Furthermore, miRISC plays a role in the transcriptional regulation of a variety of life activities, including cell proliferation, differentiation, development and apoptosis (11).

A large number of nuclear transport receptor proteins are directly involved in the process of miRNA import to the nucleus (36). Importin 8 and exportin 1, importin family members that can recognize nuclear localization sequences in protein cargoes and assist their active transport through the nuclear pore complex, have been found to be involved in the nuclear and cytoplasmic shuttling of miRNAs and RNA interference/RNA activation (RNAa) components (37,38). To function in the nucleus, miRNAs must first form an AGO-miRNA complex with the AGO protein, then bind to importin 8 and TNRC6A (also known as GW182) in the cytoplasmic-processing body (P body) for introduction into the nucleus (35,36). Unlike other members of the cytoplasmic RISC, AGO2 lacks a nuclear localization signal. TNRC6A also contains a nuclear localization signal (NLS) and a nuclear export signal (NES), which can be transported between the cytoplasm and the nucleus by importin 8 or exportin 1 (39,40). As a result, by combining with TNRC6A, which possesses NLS, AGO2 can function as a nuclear-cytoplasmic shuttling protein to re-localize the miRNA to the nucleus. TNRC6A functions as a scaffold in the nucleus, allowing other effector proteins to be recruited (41). TNRC6A directs AGO-miRNA to promoter region of target the gene, eventually cleaving the target RNA or retaining it in the nucleus. As a result, TNRC6A is yet another core protein involved in miRNA-mediated gene transcription (42,43). Furthermore, exportin 1 can transport AGO-TNRC6 complexes back to the cytoplasm. Consequently, inhibiting exportin 1 leads to an accumulation of TNRC6A and AGO2 in the nucleus (44). DICER and TRBP, on the other hand, are not attached to the RISC during their import into the nucleus. Kalantari *et al* (45) used semi-quantitative mass spectrometry to confirm that the association of AGO2 with GW182/TNRC6 and AGO3 was most well preserved and more stable in both the nuclei and cytoplasm. The interactions of AGO2 with DICER and TRBP, on the other hand, are restricted to the cytoplasm. Although cytoplasm-processed miRNAs can be introduced into the nucleus to regulate gene expression, the time frame and mechanisms through which miRNAs perform their functions in the nucleus remain to be fully elucidated.

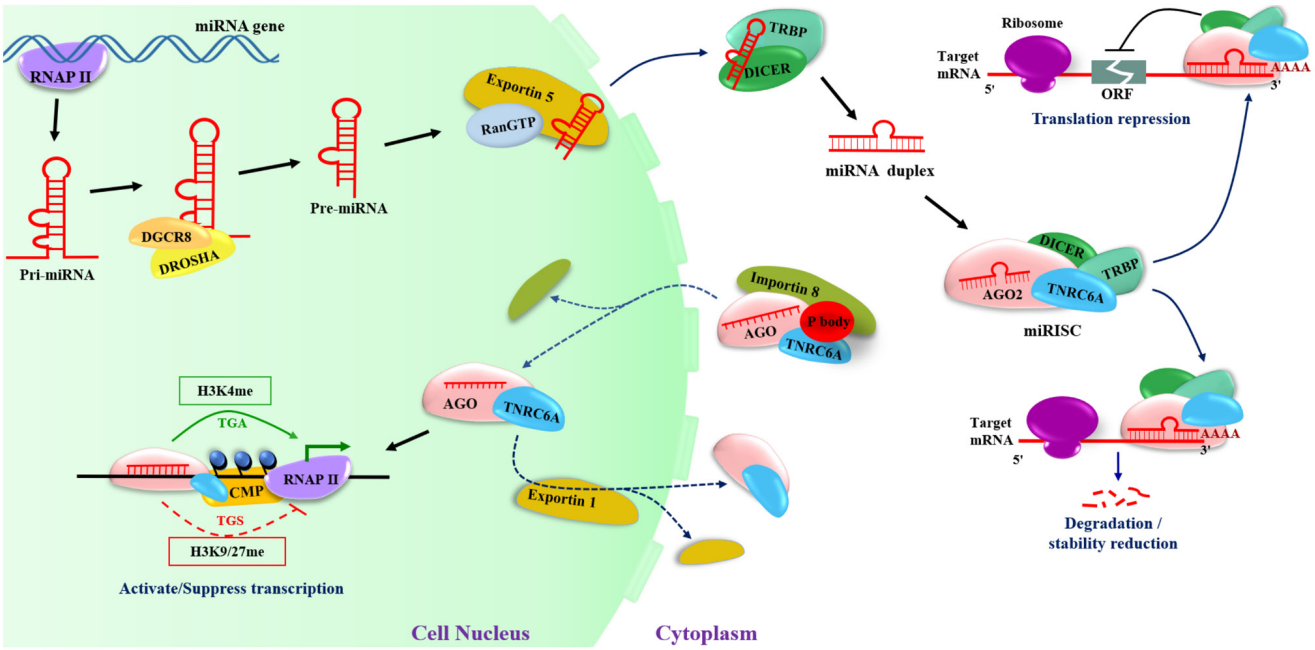

Figure 1. Biogenesis of miRNAs and their return to the nuclei. miRNAs, microRNAs; RNAP II, RNA polymerase II; pri-mRNA, primary miRNA transcript; pre-miRNAs, precursor miRNAs; H3K, histone H3; CMP, chromatin-modifying protein; AGO, Argonaute; TNRC6A, trinucleotide repeat containing 6; TRBP, TAR-RNA-binding protein; ORF, open reading frame; miRISC, miRNA-induced silencing complex.

3. Argonaute protein family and its structural characteristics

AGO proteins are members of the Argonaute family, which is highly specialized and conserved (46). In several organisms, they are the primary participants in the small non-coding RNA-mediated gene silencing pathway, and they are primarily responsible for regulating gene expression (47). The Argonaute family is divided into two groups: The ubiquitously expressed AGO subfamily proteins, also known as the AGO clade, and the gonad-expressed PIWI subfamily proteins, also known as the PIWI clade (48). AGO subfamily proteins are typically associated with miRNAs and short-interfering RNAs (siRNAs) and collaborate in the process of post-transcriptional gene silencing (49). The PIWI clade proteins are only found in another type of small RNA known as PIWI-interacting RNA (piRNA). Unlike the AGO clade, PIWI proteins are primarily expressed in germ cells and help to silence male germline transposons by interacting with piRNAs (50).

The majority of AGO proteins have 700-1,000 amino acid residues and have molecular weights of ~100 kDa, with 80% amino acid sequence identity (51). AGO protein amino acid sequences are highly conserved across species (Fig. 2A). The AGO proteins have an amino-terminal (N), a PIWI/Argonaute/Zwille (PAZ) domain, a middle (MID) domain and a PIWI domain (52-54). It should be noted, however, that the N-terminal domain is required for small RNA loading and double-stranded small RNA unwinding (55). Mammalian cells contain four AGO proteins (AGO1-4), all of which are loaded with siRNA and miRNA duplexes (56). They have similar biochemical preferences for binding to duplex RNA (45,57), but only AGO2 cleaves completely complementary mRNA targets (58).

AGO proteins have been previously shown to play critical roles in germ cell maintenance and division, as well as in transcriptional and translational regulation (59). These proteins are also involved in the repair of double-strand breaks in DNA through homologous recombination, chromatin modifications and alternative splicing. The AGO proteins, on the other hand, are incapable of catalyzing any reaction on their own. They are the core component of the RISC, interacting directly or indirectly with various partners, such as DICER, TRBP and GW182 family proteins, which are responsible for binding to short RNA and the cleavage or inhibition of target mRNA translation (60). A growing body of evidence suggests that AGO not only functions as a critical regulator of gene expression in the cytoplasm, but also mediates the repression of miRNA transcription in the nucleus, with importin 8 acting as an essential co-factor and assisting in the localization of AGO2 in the nucleus. miRNAs have also been found to colocalize in the nucleus with the AGO-TNRC6A complex and to exhibit gene-silencing activity (39). AGO1 and AGO2 proteins, in particular, promote histone H3 lysine 9 (H3K9) methylation and play critical roles in miRNA-mediated gene regulation (61). Notably, while previous research provided knowledge of the nuclear functions of AGO clade proteins, the mechanisms underlying the transport of cytoplasmic AGO proteins into the nucleus remain largely unknown.

Furthermore, the association of AGO-bound miRISC with various cellular organelles or structures remains unknown. Data on the cellular localization of the numerous steps involved in the assembly, function and recycling of these complexes are limited. It is well understood that AGO-mediated epigenetic modification is a common phenomenon that plays a role in the transcriptional regulation of nuclear miRNAs. However, further and more in-depth knowledge of the types and quantities of modified proteins recruited by the AGO complex, such as epigenetic enzymes and transcription factors (TFs) is required.

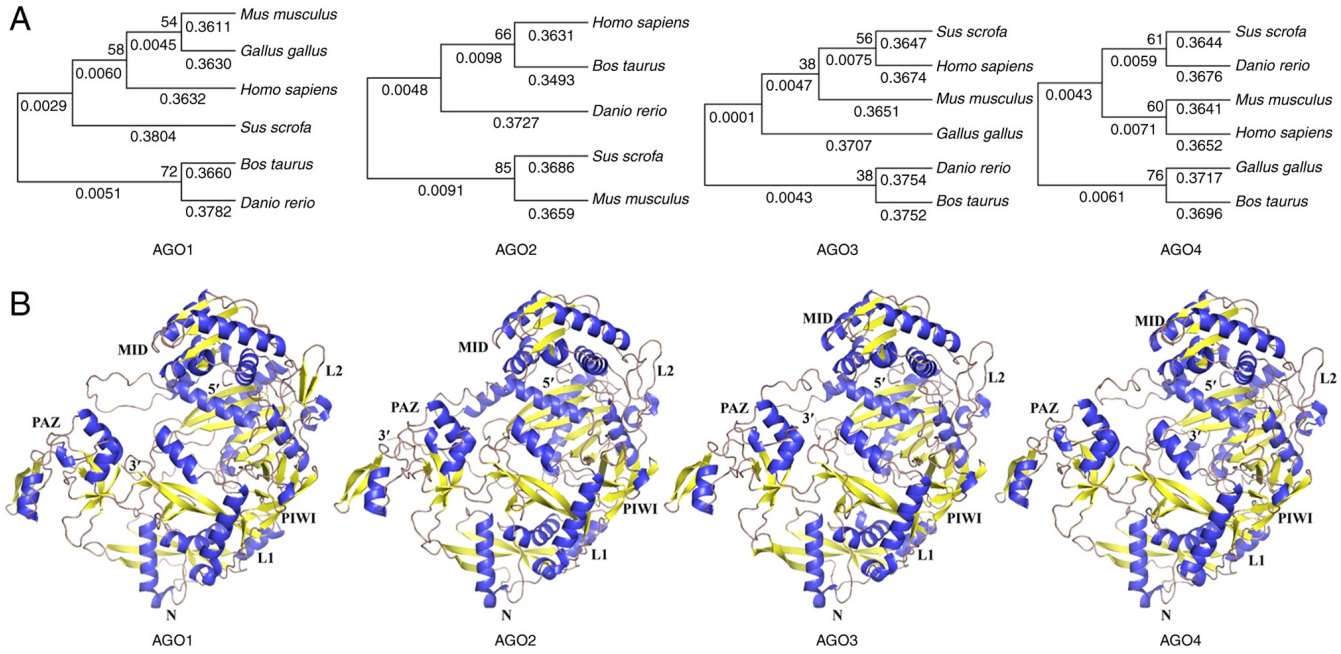

Figure 2. Type and structures of the AGO protein family. (A) AGO family conservation analysis. (B) AGO protein structure analysis. AGO, Argonaute; PAZ, PIWI/Argonaute/Zwille domain; MID, middle domain.

4. Identification and prediction of nuclear miRNAs

A number of mature miRNAs have been found to be enriched in the nucleus using high-throughput profiling technologies, such as microarray and deep sequencing. The number and types of nuclear miRNAs found in different species, tissues and cells are also distinct, emphasizing the specificity and importance of nuclear miRNA regulation (Table I).

The database RNALocate (http://www.rna-society.org/rnalocate/) is the first database which comprehensively focuses on RNA subcellular localization. miRNA symbols from the miRBase database are also provided in RNALocate. This database also includes some miRNAs with nuclear localization (62). Other databases, such as microPIR2 (https://tools4mirs.org/software/mirna_databases/micropir2/) (63), Tools4miRs (https://tools4mirs.org/) (64) and miRWalk2.0 (http://zmf.umm.uni-heidelberg.de/mirwalk2) (65) provide predicted and validated information of miRNA-target interaction. Furthermore, the program mirSTP (miRNA transcription Start sites Tracking Program) was created to predict the transcription start sites (TSSs) of miRNAs. mirSTP can be found at http://bioinfo.vanderbilt.edu/mirSTP/ (66). The knowledge of nuclear-localized miRNAs is critical for locating the core promoters of miRNAs and integrating the control of miRNA transcription into complex regulatory networks.

In Table I, a list of various nuclear miRNAs identified in various studies (35,67-74) is presented.

5. Transcriptional regulation by nuclear miRNA-mediated epigenetic modification

The mechanism of nuclear miRNA-mediated transcription regulation is similar to that of piRNA/PIWI-mediated transposon silencing (75). piRNAs assemble with PIWI members of AGO proteins to form piRNA-induced RNA silencing complexes (piRISCs) and modulate key signaling pathways at the transcriptional or post-transcriptional level in the piRNA/PIWI pathway (76). Studies have demonstrated that the nuclear PIWI-piRNA complexes at the cytoplasmic PIWI proteins silence their targets post-transcriptionally via piRNA-directed cleavage and the transcriptional ping-pong cycle. PIWI is translocated to the nucleus after piRISC assembly and represses transposons co-transcriptionally by inducing the formation of local heterochromatin at target transposon loci (77). CG9754 is a critical downstream pathway protein of PIWI/piRNA that works with PIWI to repress transposon transcription (78). Its effect on PIWI/piRNA-guided transcriptional silencing is realized after histone H3K9 is methylated as a result of its binding to the target DNA or RNA and the inhibition of genetic transposition by PIWI (79). The overexpression of the PIWI/piRNA pathway in tumor cells can lead to dysregulated methylation at H3K9, and some tumor suppressor genes may be inhibited (80). Similarly, the transcriptional activation or inhibition of genes by nuclear miRNAs is primarily accomplished through the mediation of epigenetic modifications such as H3K9 methylation and H3 lysine 4 (H3K4) acetylation. AGO proteins, in particular, as well as proteins from the epigenetic machinery, alter the structure of chromatin (Fig. 3). This process is critical for tumor cell growth, proliferation, invasion and migration, which has become a hot topic in tumor regulation research.

Following maturation, the majority of miRNAs are imported back into the nucleus, according to sequencing and bioinformatics analyses (81). miRNAs guide AGO proteins to promoter targets (82), such as the TATA-box motif or regions associated with transcription factors, owing to an abundance of putative miRNA target sites in gene promoters (83,84). The recruitment of AGO proteins (primarily AGO2) and the enrichment of RNAP II at regulated gene promoters then

Table I. Identification of nuclear miRNAs.

Tissue/cell	Method	Number of nuclear miRNAs	Highly expressed miRNAs	(Refs.)
Neural stem	ChIP/RT-qPCR	21	miR-16 miR-30c	(67)
Mouse hemopoietic	ChIP/RT-qPCR	4	miR-709/706 miR-690/467a*	(68)
HeLa	MicroRNA sequencing	346	miR-30a-5p miR-191-5p miR-148a-3p	(35)
HeLa	RT-qPCR profiling	750	miR-484 miR-191	(69)
Human megakaryocyte	Perl script	21	miR-19a-3p miR-197-5p miR-30c	(70)
Human nasopharyngeal carcinoma	Deep sequencing	339	miR-29b miR-32 miR-148a miR-148b	(71)
Rat myoblasts	Microarray assay/RT-qPCR	5	miR-340-5p miR-351 miR-494	(72)
Mouse livers	miRNA microarray assay	44	miR-30e miR-709 miR-690	(73)
Pig granulosa	Digital droplet RT-PCR	12	miR-122 miR-142-5p miR-192 miR-378	(74)

alter histone epigenetic modification and transcription factor binding. During the initiation of transcription, the DNA double helix unwinds. miRNAs typically mediate acetylation at H3K9 and histone H3 lysine 27 (H3K27), as well as methylation at H3K4, which stabilizes the promoter region and increases the recognition and binding efficiency of RNAP II, thereby activating gene transcription (85-87). Some miRNAs, such as miR-26a-1, miRNA-558, miR-195-5p and miR-24-1, have been shown to induce target gene expression. Recent research, for example, has demonstrated that nucleus-enriched miR-195-5p may regulate Foxo3 expression in porcine ovarian granulosa cells, thereby regulating follicular development and atresia. The mechanism of action is considered to involve miR-195-5p binding to the Foxo3 promoter TATA box, transcription activation, histone acetylation and hypomethylation and transcriptional regulation. Of note, it was found that AGO2, rather than other AGO proteins, regulates the effect of miR-195-5p on Foxo3 transcription (88). However, PcG protein recruitment increases H3K27 trimethylation (H3K27 me3) and decreases H3K4 trimethylation (H3K4 me3), and DNA methylation is frequently observed at some miRNA-targeted promoter regions. The chromatin structure would then be altered, and the promoter region would be tightened to establish a non-permissive transcriptional status (89,90). Several studies have found that nuclear miRNAs exert an inhibitory effect on transcriptional regulation. The lysosome is the critical organelle of autophagy, according to a study on autophagy and lysosome biogenesis, and the TF EB (TFEB) is the primary regulator of lysosome biogenesis. Dephosphorylated TFEB enters the nucleus and binds to the palindromic sequence (GTCACGTGAC) at the promoter of autophagy and lysosomal biogenesis-related genes, i.e., the coordinated lysosomal expression and regulation (CLEAR) element, to activate transcription. The miR-30b-5 represses the transcription of TFEB-dependent downstream genes by targeting CLEAR, the TFEB binding motif, further inhibiting the flux of autophagy and lysosome biogenesis (91).

miRNAs are now known to be potent gene regulators. However, there is a limited understanding of the time frame and mechanisms through which miRNAs regulate transcription (92). Several *in vitro* studies have been conducted over the past few decades, which have involved the transfection of pre-miRNAs or mature mRNA mimics into immortalized and cancer cell lines. However, further research is required to determine how well these findings reflect endogenous miRNA functions *in vivo*. Emerging evidence regarding miRNAs has demonstrated the significance of the regulatory mechanisms of nuclear miRNA expression in cancer (93,94). The critical role of nuclear miRNAs in tumor initiation, growth and metastasis is discussed below.

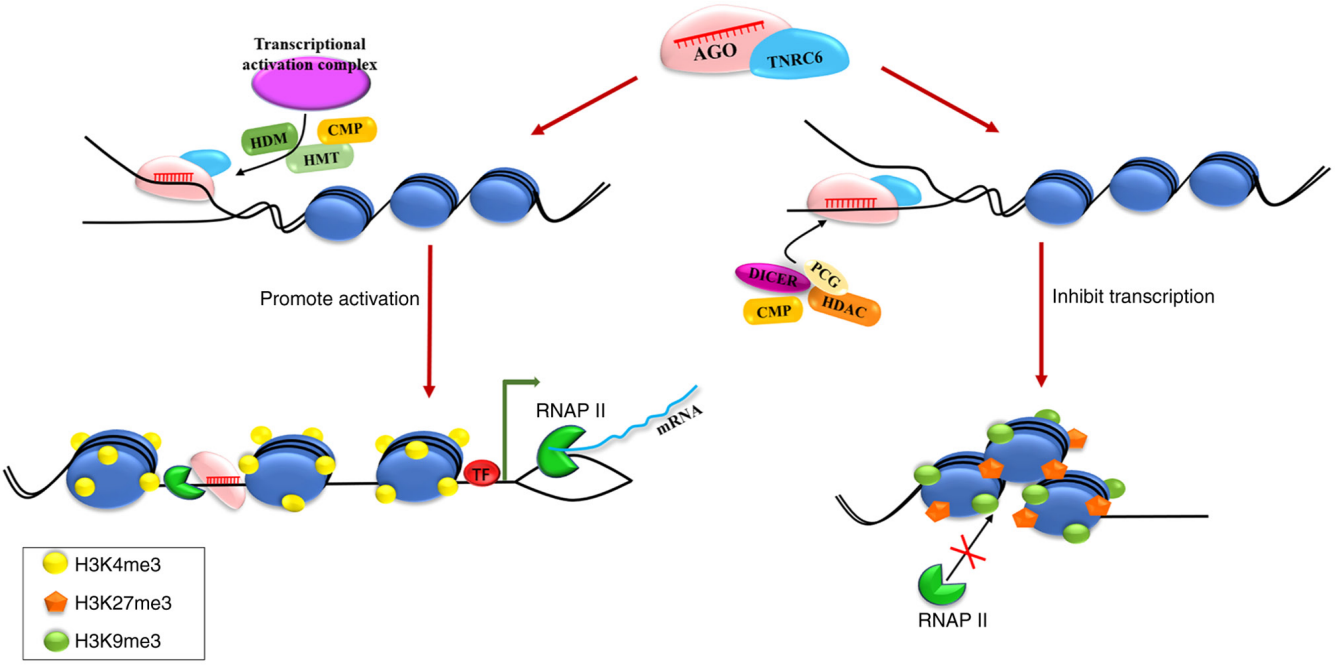

Figure 3. Transcriptional regulatory mechanisms of nuclear microRNAs. H3K, histone H3; CMP, chromatin-modifying protein; HDM, histone demethylase; HMT, histone methyltransferase; AGO, Argonaute; TNRC6, trinucleotide repeat containing 6; HDAC, histone deacetylase; RNAP II, RNA polymerase II.

6. Role of nuclear miRNAs in the transcriptional regulation of tumor cells

Previous research has suggested that changes in miRNA expression and function may contribute to cancer formation and progression, as well as to cellular responses to various types of stress (95,96). The Let-7 family, for example, influences tumor differentiation, migration, invasion and proliferation, and its members are dysregulated in various types of cancer. They can be activated or deactivated, and they can function as tumor suppressors or oncogenes (97). miR-124 has the potential to be a potent tumor suppressor in cancers with a high myc expression. A previous study discovered that miR-124 had an RNA-activating function, which resulted in the direct induction of p27 protein levels by binding to and inducing transcription on the p27 promoter region, causing G1 arrest. In breast and ovarian cancers, miR-124 can not only reverse the myc/p27/phospho-Rb protein signature to target cell proliferation, but it can also block integrin 1 and increase sensitivity to etoposide (98). Another family of onco-suppressor miRNAs, miR-34, may target cell cycle-related genes (including BCL2, MET, MYC, AXL and CDK4) and has tumor-suppressive properties that mediate apoptosis, cell cycle arrest and senescence. miR-34a, in particular, has been shown to be a direct transcriptional target of p53, which is frequently mutated in epithelial ovarian carcinomas (99,100). Furthermore, the miR-21 and miR-17-92 clusters are potent carcinogenic factors. miR-21 is a robust modulator of tumor behavior and malignant transformation. The expression of miR-21 in non-small cell lung cancer (NSCLC) tissues was regulated by the transcriptional factor nuclear factor (NF)-B binding to its promoter element. As a result, inhibiting NF-B via RNA silencing protects cells from cisplatin by decreasing miR-21 expression (101). Clusters of miR-17-92, on the other hand, are linked to cell proliferation, migration and angiogenesis. According to the findings of previous studies, miR-17-5p may inhibit the proliferation and induce the apoptosis of NSCLC H460 cells by targeting TGFR2 (102,103).

A number of factors, including matrix metalloproteinase 14 (MMP-14), circular RNAs (circRNAs_ and Cockayne syndrome protein B (CSB), play a role in the miRNA-mediated regulation of tumorigenesis (26,104,105). MMP-14, a membrane-anchored MMP that promotes tumorigenesis and aggressiveness, has been found to be highly expressed in neuroblastoma (NB) and gastric cancer. Xiang *et al* (26) identified one binding site of miRNA-584-5p (miR-584-5p) within the MMP-14 promoter through mining computational algorithm program and Argonaute-chromosome interaction dataset. Their results indicate that promoter-targeting miR-584-5p exerts tumor suppressive functions in NB through repressing the transcription of MMP-14. Zheng *et al* (106) identified adjacent binding sites of the myeloid zinc finger 1 (MZF1) and miRNA-337-3p (miR-337-3p) in the MMP-14 promoter by mining computational algorithm programs and chromatin immunoprecipitation datasets. According to the findings, miR-337-3p binds directly to the MMP-14 promoter to repress MZF1-mediated MMP-14 expression, thereby suppressing GC progression (106). circ-PLEKHM3 was identified as one of the most significantly downregulated circRNAs in ovarian cancer tissues by Zhang *et al* (107). circPLEKHM3 overexpression was found to inhibit cell growth, migration and epithelial-mesenchymal transition (EMT). Mechanistically, when circPLEKHM3 was downregulated in ovarian cancer, the competitive binding of miR-9 was attenuated, resulting in a decrease in miR-9-targeted gene expression, which promoted tumor cell proliferation (107). It has been have demonstrated that the binding sites of Let-7 and miR-29 in the proximal 3'UTR of CSB are highly conserved. Let-7 and miR-29

Table II. Advances in the transcriptional activation of miRNAs.

miRNA	Type of cell	Type of AGO	Target location	Mechanism	(Refs.)
miR-373	Prostate cancer	AGO2	*E-cadherin* promoter/ *CSDC2* promoter	/	(25)
miR-24-1	293T	AGO2	*FBP1* and *FANCC* enhancer	Increase in H3K27ac/H3K4me1 modification and p300/CBP combination; decrease in H3K9me3 modification	(86)
miR-589	Lung adenocarcinoma	AGO2	*COX-2* promoter	TFs are recruited	(112)
miRNA-558	Neuroblastoma	AGO1	*HPSE* promoter	Increase in H3K4me3 modification and decrease in H3K9me2/H3K27me3 modification	(115)
miR-6734	Colorectal cancer	/	*p21* promoter	Increase in H2B and H3 acetylation modification and decrease in H3K9me2 modification	(126)
miR-H3	$CD4^+$ T-lymphocytes	/	*HIV-15' LTR*	Pol II pre-initiation complexes (PICs) are assembled at TATA box	(127)
miR-877-3p	Bladder cancer	/	*p16* promoter	/	(128)
miR-195-5p	Porcine granulosa	AGO2	*Foxo3* promoter	Activation of RNA polymerase II initiates transcription or epigenetic modification	(88)

The forward slash symbol '/' indicates unknown information. AGO, Argonaute; H3K, histone H3; Pol II, polymerase II; FBP1, fructose-bisphosphatase 1; FANCC, FA complementation group C; COX-2, cyclooxygenase 2; HPSE, heparanase.

suppress CSB expression by directly targeting the 3'UTR of CSB in lung cancer cells, resulting in NSCLC cell apoptosis (104). The importance of the regulatory mechanisms of nuclear miRNA expression in cancer has been demonstrated by emerging evidence exhibiting genetic and epigenetic dysregulations of the machinery of miRNA biogenesis and the regulatory mechanisms of miRNAs.

Nuclear miRNAs activate transcriptional regulation in tumor cells. Under certain conditions, nuclear miRNAs have been shown to activate gene transcription and increase the expression of target genes. The human miR-373, which induces gene transcription by targeting the E-cadherin (CDH1) and cold-shock domain-containing protein 2 (CSDC2) promoters, was the first to be identified as an activator of gene transcription (25).

Nuclear miRNAs typically act at the promoter site to achieve molecular regulation. It has been reported, have found that RNAP II is enriched at the E-cadherin and CSDC2 promoters following miR-373 transfection (25). Furthermore, there is direct evidence that RISC interacts with miRNA targets in the nucleus, allowing for miRNA enrichment. Christofides *et al* (108) found that miR-548c-5p is highly enriched in human podocyte nuclei and can promote podocyte differentiation. The RISC complex, which is guided by miR-548c-5p, interacts with the promoter region of the gene to increase FOXC2 expression. There is evidence to suggest that miRNAs may function as nucleoplasmic gene regulators via a mechanism other than classic post-transcriptional repression. The nuclear miRNA-mediated regulation of gene transcriptional activation contradicts previously discovered miRNA functions involving only the degradation of genes and the inhibition of gene expression (25,109,110). Deep sequencing analysis has revealed that AGO2 is responsible for the majority of miRNAs entering the nucleus (Table II). AGO can recruit TFs and epigenetic enzymes to the promoter region of a gene, such as chromatin-modifying protein (CMP), histone demethylase and histone methyltransferase (111,112), to initiate histone modification and activate gene transcription (Fig. 3). For example, miR-589 has been shown to bind to the cyclooxygenase-2 (COX-2) promoter RNA and activate transcription. Specifically, the recognition of the promoter RNA by the miR-589-AGO2-GW182 complex may result in the recruitment of other factors, such as WDR5 and the activation of COX-2 and phospholipase A2 group IVA (PLA2G4A) gene expression (112). Turner *et al* (113) also provided the first *in vivo* evidence of RNAa by an endogenous miRNA in *Caenorhabditis elegans*. It has also been demonstrated that miRNA lin-4 can attract RNAP II to its promoter region to initiate transcription. This lin-4 miRNA overexpression is sufficient for autoactivation (113,114).

Nuclear miRNAs are currently being studied in cancer therapeutics and tumorigenesis research, in addition to

Table III. Advances in the transcriptional repression of miRNAs.

miRNA	Type of cell	Type of AGO	Target location	Mechanism	(Refs.)
miR-10a	Breast cancer	AGO1/AGO3	*HOXD4* promoter	Increase in DNA methylation and H3K27me3 modification	(125)
miR-320	Cervical carcinoma	AGO1	*POLR3D* promoter	EZH2 binding and increase in H3K27me3 modification	(129)
miR-423-5p	Breast cancer	AGO2	*PR* promoter	Increase in H3K9me2 modification	(61)
miR-939	Neuroblastoma	/	*NF-κB*	/	(130)
miR-584-5p	Neuroblastoma	AGO2	*MMP-14* promoter	Promote enhancer enrichment of zeste homolog 2 and the modification of H3K9me2/H3K27me3	(26)
miRNA-337-3p	Gastric cancer	AGO2	*MMP-14* promoter	Increase in H3K9me2/H3K27me3 modification and decrease in H3K4me3 modification	(106)
miR-223	Myeloid progenitors	AGO1/AGO2	*NFI-A* promoter	Increased levels of H3K4me3 activation marks and decreased H3K27me3 repressive marks	(131)
miR-130a	Astrocytes	/	*AQP4 M1* promoter	/	(132)
miR-552	Hepatoma	/	*CYP2E1* promoter	Inhibited Pol II binding to the CYP2E1	(133)

The forward slash symbol '/' indicates unknown information. AGO, Argonaute; H3K, histone H3; Pol II, polymerase II. HOXD4, homeobox D4; POLR3D, RNA polymerase III subunit D; MMP-14, matrix metalloproteinase 14; NFI-A, nuclear factor IA; AQP4, aquaporin-4; CYP2E1, cytochrome P450 family 2 subfamily E member 1.

regulating gene transcription activation as previously described (110-112). As previously demonstrated, in NB, AGO1 interacts with RNAP II at active promoters throughout the genome. Through the binding site in the promoter, miR-558 induces the transcriptional activation of heparanase (HPSE), facilitating tumorigenesis and NB aggressiveness. miR-558, in an AGO1-dependent manner, induces the enrichment of the active epigenetic marker and RNA pol II on the HPSE promoter in NB cells, which is inhibited by repressing the miR-558-promoter interaction (115). Huang *et al* (111) discovered that several miRNAs (e.g., miR-370, miR-1180 and miR-1236) with predicted target sites in the Ccnb1 promoter activated the expression of cyclin B1 (CCNB1) in mouse cells and influenced tumor development or growth *in vivo*. AGO1 transports miR-744 to the CCNB1 promoter region, where it promotes RNAP II and H3K4 me3 trimethylation at the CCNB1 TSS. Ccnb1 overexpression has been shown to promote tumorigenesis and to function as a putative oncogene in a variety of cancers (111,116). Wang *et al* (116) discovered that miR-370, miR-1180 and miR-1236 bound to the P21 promoter and upregulated p21 transcription in human bladder cancer cells. p21 expression attenuated the cell cycle of bladder cancer cells, preventing invasion and metastasis (116).

Of note, some enhancer markers, such as H3K27ac, are found in miRNA genes. miRNAs, according to Zou *et al* (117), interact at genomic loci, where enhancer-derived RNA (eRNA) is transcribed, and increase the mRNA levels of adjacent genes by promoting a transcriptionally active chromatin state. The overexpression of miR-24-1, for example, in 293T cells can increase the expression of its neighboring genes, FBP1 and FANC. Increased levels of miR-24-1 will result in the enrichment of RNA polymerase II, p300/CBP and enhancer RNAs, as well as an increase in histone H3K27ac/H3K4 me1 modification and a decrease in H3K9 me3 modification (86). Notably, ChIP-qPCR has revealed that AGO2 is present at the enhancer locus (86). Furthermore, the protein-coding genes, ITGA9, CTDSPL, VILL and PLCD1, surround the miR26a-1 gene. However, when the seed region of the miRNAs is deleted or mutated, or when the enhancer locus is deleted, the activation is disrupted, suggesting that this function is dependent on the base-pairing of miRNA-enhancers.

Inhibitory effect of nuclear miRNAs on the transcriptional regulation of tumor cells. It is now clear that miRNAs regulate gene expression in mammalian somatic cells at both the transcriptional and post-transcriptional levels. Apart from post-transcriptional gene silencing via the RISC pathway in the cytoplasm, it is well established that the regulatory function of miRNAs in the nucleus is primarily achieved through transcriptional activation or inhibition of genes via epigenetic modifications such as H3K9 methylation and H3K4 acetylation (118). Following miRNA transport into the nuclei, AGO proteins recruit inhibitory complexes to the miRNA-targeted promoter region (Fig. 3), which are primarily composed of RISC (AGO and DICER1 proteins), PcG elements (YY1, EZH2 and SUZ12), CMPs and histone deacetylase. This interaction allows the protein inhibitor complex to move closer to the targeted promoter region, causing an increase in H3K27 me3 modifications and a decrease in H3K4 me3 modifications, altering the chromatin structure and establishing a non-permissive transcriptional status (119). Some examples of the regulatory mechanisms

of nuclear miRNA-mediated transcriptional repression are presented in Table III.

Studies revealing the epigenetic dysregulations of nuclear miRNAs, biogenesis-based machinery and nuclear miRNA regulatory mechanisms have demonstrated the importance of miRNA expression regulation in cancer (120). This may lead to the discovery of novel predictive markers for the prognosis of cancer patients. For example, in gastric cancer, Guo *et al* (121) demonstrated for the first time, using a dual-luciferase report system, that YWHAZ was a target gene of miR-375 and played a role in miR-375 regulation in the malignant phenotype of gastric cancer. However, the precise mechanism through which YWHAZ regulates gastric cancer cell migration and invasion remains unknown. The overexpression of miR-375 was shown to inhibit gastric cancer cell migration invasion, EMT, and the activation of the Wnt/β-catenin signaling pathway, indicating that the miR-375/YWHAZ axis may be a novel therapeutic target for gastric cancer (121). Similarly, Liu *et al* (22) found that miR-675 aided gastric cancer cell proliferation and invasion by targeting the paired-like homeodomain transcription factor 1 (PITX1) and promoting EMT and the activation of the Wnt/β-catenin signaling pathway. Furthermore, the role of miR-652 as an oncogene in gastric cancer in targeting the RORA pathway can promote gastric cancer cell proliferation, migration and invasion and may be a novel predictive marker for the poor prognosis of patients with gastric cancer (122). Furthermore, the role of miR-652 in targeting the VEGF pathway can impede the formation of vascular networks and may be used to develop anti-angiogenetic agents. The molecular mechanisms of nuclear miRNAs regulating the occurrence of cancer have been explained using newly developed techniques and more detailed exploration.

Majid *et al* (23) used miRNA-205 to transfect three human PCa cell lines (LNCaP, PC3 and Du145), as well as a non-malignant epithelial PCa cell line (RWPE-1). Their findings revealed that miR-205 functioned as a tumor suppressor miRNA in Pca by directly targeting the tumor suppressor genes, IL24 and IL32, via the apoptotic and cell-survival pathways, and that its overexpression easily induced the expression of both genes. Further investigations revealed that miR-205 directly targeted tumor suppressor genes (IL-24 and IL-32) to suppress tumorigenesis via active histone modifications, such as 2H3K4, 3H3K4, the acetylation of Lys 9/14 of histone 3 and pol II (23). miR-584-5p has been shown to inhibit MMP-14 expression in NB cells. AGO2 has been shown to transport miR-584-5p to bind to the MMP-14 promoter, promoting EZH2 enrichment and the methylation of H3K27 and H3K9. It has also been shown to prevent MMP-14 transcription from suppressing NB cell growth, migration, invasion and angiogenesis (26). ABCG2 repression has been linked to aberrant promoter methylation in a certain type of cancer, including renal carcinoma and multiple myeloma. In colorectal cancer (CRC), the *de novo* DNA methyltransferase (DNMT3B) is a direct target of miR-203. Notably, miR-203 expression in CRC can be downregulated by reducing DNMT3B repression, resulting in a lower miR-203 expression in CRC and causing methylation of the ABCG2 promoter, which significantly inhibits ABCG2 expression and promotes cancer development (123). Notably, miR-215-5p has been found to target both the PCDH9 gene promoter and the mRNA 3'UTR, inhibiting PCDH9 expression, while promoting glioma cell growth and proliferation (124). Furthermore, while several studies have demonstrated the oncogenic or tumor-suppressing roles of nuclear miRNAs, there are some unresolved issues regarding the possible regulatory mechanisms modulating nuclear miRNA expression in cancers.

7. Conclusions and future perspectives

miRNAs have been shown to be extensively deregulated in human cancers over the past few decades, highlighting their critical role in tumor initiation, growth and metastasis. Similarly, the regulatory mechanisms that control miRNA expression are strongly linked to cancer diagnosis, prognosis and treatment, as well as to cancer pathogenesis. miRNAs typically suppress target gene expression by partially binding to complementary sequences in the 3'-UTR of target mRNAs, inhibiting translation and degrading the target mRNAs. However, additional evidence suggests that miRNA-mediated gene expression regulation also has other functions, and the 5'-UTR coding sequence or promoter of the genes could be the target region. Several small RNAs within miRNA response elements, such as circRNAs and long non-coding RNAs, have been shown to interact with miRNAs to regulate target gene expression. Current research on the regulation of miRNAs, however, is still focused on the traditional cytoplasmic pathways and how miRNAs specifically control specific types of cancer. The present review article summarized the series of processes involved in the entry of miRNAs into the nucleus, particularly new mechanisms of nuclear miRNA expression regulation in tumors. Mature miRNAs that are carried into the nucleus and the target gene promoter region by AGO proteins to activate epigenetic modification and regulate transcription perform the majority of the regulatory functions of miRNAs in the nucleus. A better understanding of nuclear miRNA regulation and the potential underlying mechanisms may aid in the understanding of the association between miRNAs and tumorigenesis. These new signs of progress will lead to the development of more precise therapeutic targets and the identification of novel biological markers for the diagnosis of tumors and cancers, as well as new avenues for future research on a variety of malignant diseases.

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Authors' contributions

JL was mainly responsible for collecting all relevant information and completing the review article. TY was were mainly responsible for performing the literature search. ZH and HC

were mainly responsible for revising the manuscript. YB was responsible for the conception of the review and the assignment of tasks. There was no additional assistance with manuscript preparation. All authors have read and approved the final manuscript. Data authentication is not applicable.

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Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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