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Review

Nuclear miRNAs: Gene Regulation Activities

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Abstract: MicroRNAs are small non-coding RNAs, which contribute to the regulation of many physiological and pathological processes. Conventionally, miRNAs perform their activity in the cytoplasm, where they regulate gene expression by interacting in a sequence-specific manner with mature messenger RNAs. Recent studies point to the presence of mature miRNAs in the nucleus. This review summarizes current findings regarding the molecular activities of nuclear miRNAs. These molecules can regulate gene expression at the transcriptional level by directly binding DNA on the promoter or the enhancer of regulated genes. MiRNAs recruit to these regions different protein complexes, resulting both in activation or repression of transcription, through a number of molecular mechanisms. Haematopoiesis is presented as a paradigmatic biological process whereby nuclear miRNAs possess a relevant regulatory role. Nuclear miRNAs may affect gene expression also acting on nuclear mRNA processing and on the biogenesis of miRNA themselves by regulating pri-miRNA maturation. Overall, nuclear miRNAs are biologically active molecules that can be critical for the fine tuning of gene expression and deserve further studies in a number of physiological and pathological conditions.

Keywords: miRNAs; nuclear localization; gene regulation; transcriptional control; RNA procesing; Haematopoiesis

1. Introduction

MiRNAs are classically regarded as negative regulators of messenger RNA (mRNA) function. This activity is mainly obtained through both the repression of mRNA translation and induction of mRNA degradation, upon the binding of miRNAs to the 3′ untranslated region (3′ UTR) of mRNAs [1,2]. These fu

nctions of microRNAs take place within cellular cytoplasm, through their binding to mature mRNAs [3]. However, a number of reports indicate that miRNAs may possess wider activities, that include the direct regulation of chromatin structure and transcription [4,5]. This type of regulatory activity could be activating or repressing, depending on the protein complexes that miRNAs drive to chromatin DNA. Therefore, miRNAs are active within the nucleus, where they must remain or be transported back to the nuclear side of the nuclear membrane.

This review briefly summarizes current evidences indicating such non - canonical nuclear activities of miRNAs, highlighting their biological roles in the regulation of haematopoiesis.

2. Biogenesis of MicroRNAs

The biogenesis of miRNAs takes place in the nucleus and cytoplasm in several steps. (Figure 1). They are typically transcribed by RNA polymerases II or III as long primary miRNAs (pri-miRNAs) with an internal loop structure containing a double-stranded stem region and an apical loop. The pri-miRNAs are transformed into precursor miRNAs (pre-miRNAs), structures of approximately 70 nucleotides (nt), by means of a protein complex consisting of the RNase III enzyme Drosha and the DGCR8 protein (Di George syndrome critical region 8 gene) that binds double-stranded RNA (Figure 1(1)) [6–8]. The pre-miRNAs are further processed after being translocated into the cytoplasm. The

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export mechanism involves a protein complex consisting of Exportin5 (XPO5), a member of the karyopherin β family, and a GTPase (Ran GTP) [9,10] (Figure 1(1)). Pre-miRNAs in the cytoplasm are converted into mature, 22 nt duplex miRNAs by hairpin cleavage by the enzyme DICER [11]. The ds-miRNA molecule is then loaded onto the miRNA induced silencing complex (miRISC), a ribonucleoprotein complex consisting of the Argonaute (AGO1-4), DICER, TRBP and TNRC6A proteins. TRBP identifies the 'guide' and 'passenger' strands in the molecule. TRBP loads the ds-miRNAs in the correct orientation onto the AGO proteins based on their thermodynamic properties and, once it recognizes the strand with the least stable 5' end, the AGO protein duplexes and removes the passenger strand to form the mature miRNA molecule [12,13] (Figure 1(2)). The AGO protein family contains several members, among them AGO2 possesses the activity of cutting RNA molecules. AGO2 is important for miRNA maturation and for gene silencing mechanisms regulated by small RNAs [14,15]. Several previous studies have found that miRISC is imported into the nucleus of mammalian cells and that the nuclear RISC (a complex of ~158 kDa) is about 20fold smaller than its cytoplasmic counterpart (a complex of nearly 3 MDa). Western blotting analysis showed that nuclear RISC consists of various proteins, such as AGO and GW182/TNRC6 [16,17]. In the study by Kalantari et al [18] semi-quantitative mass spectrometry confirms that the association of AGO2 with GW182/TNRC6 and AGO3 is well conserved and more stable in both nucleus and cytoplasm [19]. In contrast, AGO2 interactions with DICER and TRBP are limited to the cytoplasm. The import process of miRNAs into the nucleus involves various nuclear transport receptor proteins [20,21]. It has been discovered that the nuclear and cytoplasmic movement of miRNAs is driven by importin 8 and exportin 1. These are two members of the importin family that recognize the nuclear localization sequences in proteins and perform their active transport through the nuclear pore complex [22–26].

To enter the nucleus, miRNAs must first form a complex with the AGO protein, which binds to importin 8 and TNRC6A [20,26] (Figure 1(3)). TNRC6A can move from the nucleus to the cytoplasm and back again as it possesses a nuclear localization signal (NLS) and a nuclear export signal (NES) [25,26]. Consequently, by interacting with TNRC6A, AGO2-miRNA complex enters into the nucleus. TNRC6A guides the miRNA AGO complex to the target gene, in its promoter region complementary to seed region. Thus, TNRC6A is a central protein involved in miRNA-mediated gene transcription regulation [27,28]. Furthermore, AGO-TNRC6 complex can move to the cytoplasm interacting with exportin 1 (Figure 1(4)) [29]. In contrast, DICER and TRBP enter the nucleus without being linked to RISC.

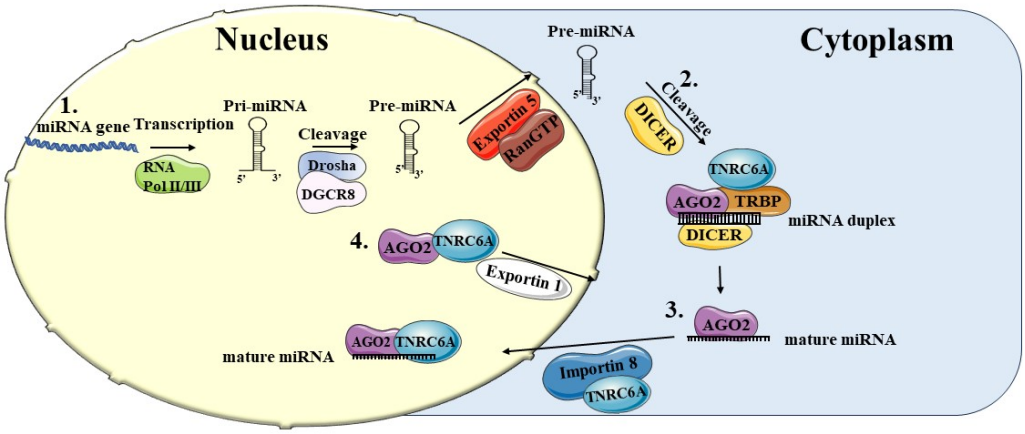

Figure 1. Biogenesis of miRNAs and their return to the nucleus. The biogenesis of miRNAs is mediated by several steps. (1) miRNAs genes are transcribed by RNA Pol II or Pol III into primary miRNAs (pri-miRNAs) and then cleaved by Drosha and DGCR8 into precursor miRNAs (pre-miRNAs). They are then exported into the cytoplasm with the help of Exportin-5 and RanGTP. (2) The pre-miRNAs are cleaved into miRNA duplexes by DICER1 in the cytoplasm and associated with the Argonaute proteins (AGO), forming the mature miRNA. (3) Part of the mature miRNAs are translocated into the nucleus with the help of Importin-8 (IPO8) and TNRC6. (4) AGO2 and TNRC6

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can be transported into the cytoplasm via Exportin 1 (also called XPO1 or chromosomal region maintenance 1 (CRM1). miRNA, microRNA; RNAPolII/III, RNA Polymerase II/III; pri-miRNA, miRNA primary transcript; pre-miRNA, miRNA precursor-; AGO2, Argonaute 2; TNRC6A, trinucleotide repeat containing 6; TRBP, transactivation response-RNA-binding protein.

3. Evidence of miRNAs in the Nucleus

Meister et al., by studying Hela cells, demonstrated that mature miR-21 can be found in the cytoplasm and in the nucleus. This discovery suggested that a number of mature miRNAs may be transported back into the nucleus, where they may exert a functional activity [30,31]. In 2009, Földes-Papp et al. used femtosecond laser microscopy to assess the association between molecular beacon probes and human single-stranded cellular targets of miR-122 [32]. They demonstrated that mature miR-122 from the cytoplasm enters the nucleus of human hepatocytes. In recent years, more and more studies have highlighted the presence of mature nuclear miRNAs in most mammalian cells thanks to high-throughput analyses [4,33]. Several studies have studied the distribution of microRNA in the cytoplasm and nucleus, taking advantage of high performance next-generation sequencing and microarrays [34,35]. A sequencing study of nuclear and cytoplasmic fractions deriving from endothelial cells subjected to hypoxia highlighted an increased localization of miR-210-3p in the nucleus. By using confocal microscopy, we studied miR-223 localization during retinoic acid-induced granulopoiesis in HL60 cells and primary blasts. In these conditions mature miR-223 translocates to the nucleus and shows an increasing nuclear pattern. Furthermore, in situ hybridization experiments showed that, following treatment with retinoic acid, miR-223 shows the ability to bind metaphase chromosomes, where RNA transcription is absent or minimal [36]. The study of the sequence of nuclear miRNAs has highlighted the existence of a nuclear localization signal (AGUGUU) at their 3′ terminal sequence [33]. Some nuclear miRNAs such as miR-193b, miR-19, miR30b, miR-30c, miR-590-5p, miR-374 and miR-374b contain very similar nuclear localisation motifs in their sequence with small variations, including UGUGUU, ACUGUU, AGAGUUU, AGUCUUU, AGUGAU, AGUGUA, AGNGUN [4,37]. It is hypothesized that these specific sequences targeting miRNAs to the nucleus are recognized by the nuclear pore structures, allowing their entry. Interestingly, the nuclear localization of a specific miRNA varies based on the tissue type. For example, miR-29b has a nuclear localization in HeLa cells but not in other cell lines [38], suggesting a tissue-specific functional role of nuclear localization. Overall, the significant number of recent data showing the nuclear localization of miRNAs, supports their functional role into the nucleus.

4. Functions of miRNAs in the Nucleus

Many functions of nuclear miRNAs have been reported in recent years, among them very important is their ability to bind complementary sequences located on gene promoters or enhancers. The action of nuclear miRNAs associates with mechanisms of epigenetic modifications of DNA or chromatin status at the targeted genomic sites, leading to transcriptional activation or repression of target genes [94]. Nuclear miRNA activity depends also on the genomic location of binding sequences and on the epigenetic status at these sites (such as presence of TATA box motifs or CpG island regions) [39]. In addition, miRNA target regions can be located far away from the transcription start site (TSS) [40].

5.1. Transcriptional Activation

Numerous studies show how miRNAs can mediate transcriptional gene activation (TGA) by interacting with the promoters (Figure 2A), or with enhancers (Figure 2B), of their target genes.

5.1.1. Interaction with Promoters

One mechanism by which miRNAs activate gene transcription could be the association with promoters and their epigenetic regulation. MiR-589 targets the promoter RNA of cyclooxygenase 2 (COX-2) by recruiting AGO2 and GW182 (TNRC6A) to form a complex. This complex can modify

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histones through association with WDR5, a protein that stimulates histone methyltransferase activity. Consequently, in the COX-2 promoter H3K4me3 and H4 acetylation levels increase, and gene transcription is activated [48] (Figure 2(A1)). FOXO3, an important gene in the regulation of ovarian follicular development and atresia, is regulated by miR-195-5p. This miRNA enters the cellular nucleus, associates with AGO2 and interacts with the FOXO3 promoter by recognizing a complementary sequence in the TATA-box (Figure 2(A2)). Consequently, histone acetylation, hypomethylation, and transcriptional activation take place [41]. Transcriptional activation markers such as acetylated H3 and H4 and H3K4me2 are enriched in the promoter of the interleukin (IL) tumor suppressor genes IL24 and IL32, at specific sites bound by miR-205. As a consequence, RNA polymerase II (Pol II) is recruited and, subsequently, transcribes the IL24 and IL32 genes [42,43]. MiR-744 and miR-1186 increase transcription of the cyclin B1 gene (Ccnb1), by interacting with sequences on this gene promoter, which are highly complementary to their respective seed regions. Moreover, miR-744 association with AGO1 causes an enrichment of Pol II and H3K4me3 marks at the Ccnb1 TSS [44].

Overall, three types of interactions have been hypothesized between miRNAs and the promoters of the genes that are regulated at the transcriptional level. One type of interaction (Figure 2(A1)) leads to the formation of a direct miRNA-DNA double helix complex. In this case, miRNA binds the promoter in association with AGO proteins and recruits histone modifiers that increase the levels of activating markers, such as H3K4me3, while decreasing the inhibitory histone marks [47]. In a second model the miRNA-AGO complex interacts directly with the TATA box motif region or sites on the promoter also bound by transcription factors (Figure 2(A2)). This binding event leads to the recruitment of TATA box-binding protein (TBP), Pol II and histone-modifying proteins such as methyltransferases or acetyltransferases, resulting in gene transcriptional activation [45,46]. Among miRNAs that act accordingly to this model there are miR-181d, let-7i and miR-138, which activate transcription of c-myc, IL-2 and insulin genes, respectively by interacting with the TATA-box on the promoter of these genes. In a last model, miRNAs bind to the promoter-associated sense or antisense RNA (pRNA), transcribed by the target gene promoter. Again, as in the other models, this interaction recruits transcription factors, histone modifiers and Pol II to transcriptionally regulate gene expression [48–50] (Figure 2(A3)).

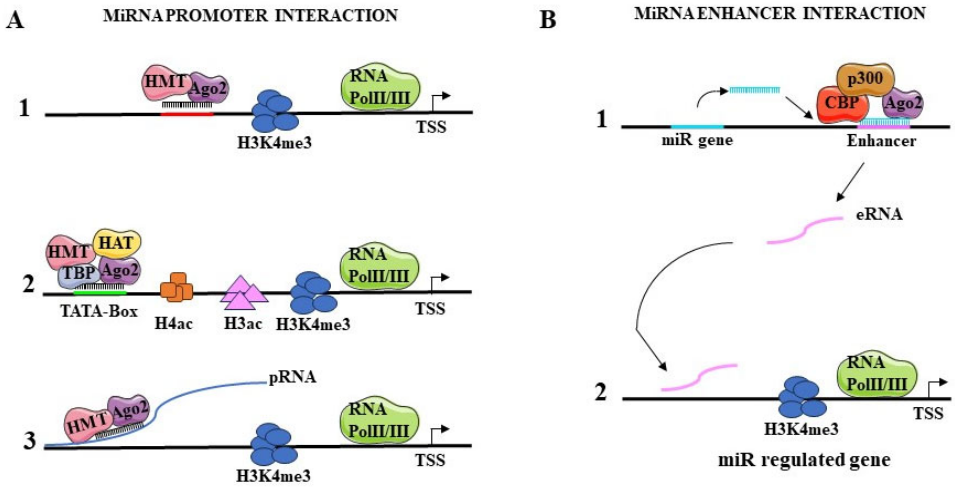

Figure 2. The activating function of nuclear miRNAs. (A) miRNA-mediated gene promoter regulation. (1) Direct interaction between miRNAs and complementary sequences on target genes promoters with the presence of AGO2. This interaction allows an activator protein complex to be located in the vicinity of the targeted promoter region, whose chromatin structure is enriched with activator markers such as H3K4me3. RNA Pol II is recruited and the gene is transcribed. (2) the miRNA-AGO-TBP complex interacts directly with the TATA box motif present on the promoter recruiting HMT and HAT. Chromatin is modified, activating markers such as H3K4me3, H3ac and

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H4ac are increased, and RNA Pol II is recruited to initiate transcription. (3) RNA Pol II transcribes the promoter into promoter-associated RNA (pRNA). AGO2 mediates miRNA binding to pRNA, recruits HMT by producing active histone modifications, such as H3K4me3 with RNA polymerase II enrichment. (B) Transcription regulation through interaction with enhancers.(1) A miRNA is transcribed from its gene located near enhancer loci. The mature miRNA forms a complex with AGO2 and p300/CBP, inducing active chromatin markers in the enhancer regions, and RNA polymerase II call. Thus, the enhancer is transcribed. (2) Next, the eRNA binds to p300 and other proteins to activate the target gene promoter. miRNAs, microRNAs; RNA Pol II, RNA polymerase II; H3K, histone H3; AGO2, Argonaute 2; TBP, TATA-BOX-binding protein; eRNA enhancer RNA; HAT, histone acetyltransferase; HMT, histone methyltransferase; pRNA, promoter-associated RNA.

5.1.2. Interaction with Enhancers

MiRNA may mediate transcriptional activation through the regulation of enhancers. In this case, miRNA binding loci are located in the proximity of enhancer regions displaying the typical histone marks of functional enhancer regions, such as H3K27ac (Figure 2B). miRNAs are transcribed and produced. Subsequently, they form a complex with AGO2 and p300/CBP, which is able to increase the levels of H3K27Ac and H3K4me1 and to decrease those of H3K27me3 on the enhancer region surrounding the miRNA locus. These histone modifications induce the interaction of Pol II with the enhancer, the recruitment of transcription factors and the expression of enhancer RNA (eRNA) (Figure 2(B1)). Once transcribed, the eRNA interacts with the promoter of nearby located genes to promote their transcription (Figure 2(B2)). The gene locus of miR-26a-1 is located near to the genes coding for the ITGA9, CTDSPL, VILL and PLCD1 proteins [51,52]. It has been shown that when miR-26a-1 is overexpressed, there is a transcriptional activation of the ITGA9 and VILL genes. The seed sequence of miR-26a-1 and the enhancer sequence of these genes shows complementarity. When the miRNA seed or the enhancer sequence of the two genes is deleted or mutated, no transcriptional activation is obtained. Similarly, an enrichment of H3K27ac, a marker of enhancer regions, is detectable nearby the genomic locus of miR-3179, inducing transcriptional activation of adjacent PKD1P and ABCC6 genes [53]. In breast cancer cells, Liang et al. observed that miR-339 interacts with the enhancer of the neighbouring gene GPER resulting in its upregulation [54]. A similar mechanism was observed for miR-24-1, that interacts with the enhancers of the neighbouring genes FBP1 and FANCC. In association with AGO2, it promotes the transcription of these genes [53]. In this model, the transcribed eRNA is ready to interact with specific sites in the promoters of the target genes. Using microarrays, several enhancers were identified that exhibit sequence complementarity with the seed region of miR-24-1. The ectopic overexpression of miR-24-1 activates distant target genes, such as the KDM6B gene, by increasing the transcription of their enhancers [53]. Thus, miRNA-induced transcriptional activation of target genes via enhancer regulation can affect the expression of genes residing on the same genomic locus as well as distantly located genes.

5.2. Transcriptional Repression

Many studies show that mature nuclear miRNAs can repress the transcription of their target genes. Among the known mechanisms that are used for this regulation is their direct interaction with the promoter through the recognition of DNA sequences highly complementary with the miRNAs seed region. We have shown that this mechanism is used by miR-223 to regulate NFI-A gene transcription during granulopoiesis. We showed that miR-223 localizes in the nucleus and binds to two evolutionarily conserved regions in the NFI-A gene promoter, which are complementary to the miR-223 seed sequence. MiR-223 recruits to that genomic region a protein complex consisting of AGO1, DICER1 and Polycomb proteins (PcG) YY1 and SUZ12, which are responsible for the trimethylation of H3K27 [55,56]. Thus, miR-223 generates an inactive chromatin state on the NFI-A gene promoter, which in turn blocks its expression. This event is required to direct the granulocytic differentiation of hematopoietic progenitors [57]. Benhamed et al. demonstrated that nuclear miRNAs of the let-7 family, in particular by let-7f, acts on the promoters of genes involved in cellular senescence and induces their transcriptional gene silencing [58]. The CDC2 and CDCA8 are genes of

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the RB1/E2F complex and are repressed during cellular senescence. The sequence of their promoters shows sites complementary to let-7f, where the association of miRNA with a protein complex consisting of AGO2, RB1 and E2F occurs. This results in the recruitment at these sites of histone methyltransferases (HMTs) and histone deacetylases (HDACs), which induce an increase in repressive H3K27me3 and H3K9me2 histone marks, paralleling a decrease in the activating H3K4me3 marks and leading to a repression of the expression of genes involved in cellular senescence. Our unpublished data on acute promyelocytic leukemia cells induced into myeloid differentiation by treatment with retinoic acid show an increased expression of let-7c, which upon this treatment translocates into the nucleus of differentiated cells. In addition, whole-genome chromatin IP sequencing identifies a number of genes that are bound by let-7c and are relevant for myeloid differentiation. These genes can be transcriptionally activated or repressed, following an accumulation in their chromatin of activating H3K4me3 or repressing H3K27me3 marks, respectively. These data further confirm that nuclear miRNAs can perform activatory and repressive transcriptional functions. In gastric cancer, miR-584-3p interacts with the promoter of MMP14 (matrix metalloproteinase 14), at an upstream region of the TSS, and represses its transcription. This regulation occurs as a result of the recruitment of a protein complex consisting of AGO2, EZH2 and EHMT2 to the promoter. This results in increased levels of H3K27me3 and H3K9me2 characteristic of inactive chromatin [59] (Figure 3(1)). Genes involved in cardiac hypertrophy are transcriptionally repressed by the interaction of miR-208b with the promoters and recruitment of EZH2. As a consequence of EZH2 binding, the chromatin of these promoters is enriched with inhibitory markers (H3K27me3) [60]. In addition, miR-320 interacts with the gene promoter of the RNA polymerase III subunit D (POLR3D), where it recruits the EZH2 and AGO1 proteins. This results in transcriptional silencing of the POLR3D gene following the accumulation of the inhibitory marker H3K27me3 [61]. A peculiar mechanism of repression is operated by miR-126-5p to support endothelial integrity and counteract atherosclerosis. MiR-126-5p in a complex with AGO2 is transported to the nucleus by means of its association with Mex3a, an RNA binding protein. Within the nucleus, AGO2 is released from miR-126-5p, which then is found associated with caspase-3. The association blocks the activity of caspase-3 since it impairs its ability to dimerize, leading to apoptosis inhibition. Thus, in this mechanism, a nuclear miRNA is able to block the function of a protein through the formation of a riboprotein complex. Biologically, this leads to the protection of endothelial cells from apoptosis in conditions of shear stress during the process of atherosclerosis [62]. Some miRNAs appear to function as transcriptional and posttranscriptional regulators of the same target gene. As described above, this concept applies to let-7 family miRNAs [58] and to the regulation of the expression of the NFI-A gene during granulopoiesis by miR-223 [57] (see below). MiR-552 also has a dual regulatory effect at both the transcriptional and post-transcriptional levels. This miRNA directly binds the CYP2E1 gene promoter, but this interaction occurs through a region of the miRNA that is not the seed region. At the same time, miR-552 performs a classical posttranscriptional regulation through the binding of the 3' UTR of CYP2E1 mRNA [63]. As mentioned above, miRNAs may regulate transcription by interacting with RNA produced by promoter sequences. Some promoters are transcribed into an antisense strand, producing a pRNA, containing sequences complementary to miRNAs [64]. The miRNA interaction with the antisense pRNA transcript occurs through the seed sequence and results in transcriptional repression of the target gene. Following this binding, an inhibitory protein complex consisting of EZH2, YY1, AGO, Dicer1 and SUZ12 is recruited to the promoter, resulting in chromatin modification and an increase in the repressor markers H3K27me3 and H3K9me2 (Figure 3(2)). An example of this regulatory pattern is the activity of miR-423-5p on the antisense transcript of the promoter of the gene coding for the progesterone receptor (PR). It has been observed that as a result of this interaction, miR-423-5p recruits AGO2 and histone modifiers that increase the level of histone H3K9me2 in the PR promoter leading to transcriptional silencing of the gene [65].

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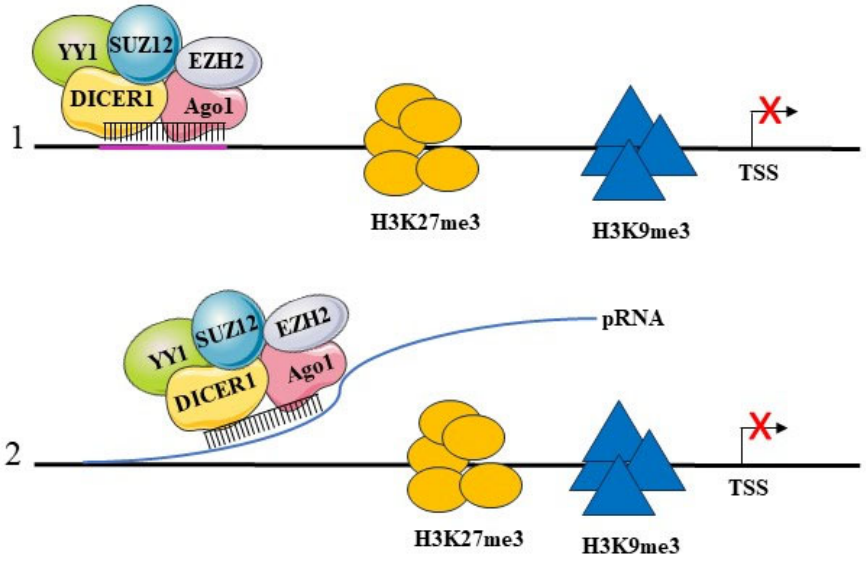

Figure 3. The suppressive function of nuclear miRNAs. MiRNAs can inhibit gene expression at transcriptional level. The miRNA-AGO1 complex recruits a protein complex consisting of YY1, DICER1, SUZ12, EZH2 that promotes inhibitory cromatin modifications with increased H3K27me3 and H3K9me3 markers. This can occur through direct binding to the promoter via sites complementary to the seed region (1) or through binding to the pRNA, a promoter associated non-coding transcript (2). In this way, miRNA leads to a decrease in RNA Pol II and the suppression of target genes. YY1, Yin Yang 1 transcription factor; EZH2, Enhancer of Zeste Homolog 2; SUZ12, Suppressor of Zeste 12 protein homolog; TSS, transcription start site, pRNA, a promoter-associated RNA.

6. Nuclear miRNAs in Hematopoiesis

Many studies have related the differential expression and activity of miRNAs with the regulation of hematopoietic differentiation [66–70]. In particular, differentiation factors, cell cycle regulators or transcription factors that are involved in hemopoiesis appear to be targets of miRNAs. By studying the expression profiles of miRNAs at different stages of hematopoietic differentiation, some of the key regulators have been identified [71–75]. Among these miRNAs, some show a predominantly nuclear localization. However, there is limited work evaluating the nuclear role of these miRNAs and their involvement in hematopoietic differentiation. We showed that miR-223 performs a dual regulation on the NFI-A gene, by virtue of both its cytoplasmic and nuclear component. The cytoplasmic component has a major regulatory role in hematopoiesis. MiR-223 transcription is repressed by NFI-A, whereas it is increased by the transcription factor cEBPalpha, which induces myeloid differentiation of granulocyte-monocyte progenitors. In turn, cytoplasmic miR-223 represses NFI-A translation [76]. Besides these activities, as described above in this review, we demonstrated that miR-223 localizes in the nucleus of hematopoietic cells, where it transcriptionally represses NFI-A gene expression to promote granulocyte differentiation [36]. In fact, the levels of miR-223 are finely regulated during hematopoiesis [78]; the transcriptional repression of miR-223 expression by the oncogenic fusion protein AML1/ETO, triggers the development of leukemia [78]. A study of the nuclear expression profiles of miRNAs during mouse granulopoiesis identified an accumulation of miR-690 in the nucleus. This miRNA targets the CEBPalpha gene; when miR-690 is overexpressed CEBPalpha is downregulated. The retention of miR-690 in the nucleus decreases the share of cytoplasmic miR-690, with consequent increased expression of CEBPalpha and myeloid differentiation. MiR-706 is also enriched in the nucleus of myeloid cells, with a decrease in its cytoplasmic localization in granulocytes compared to promyelocytes. This miRNA would appear to negatively regulate the STAT1 transcription factor, which is important for myeloid differentiation

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[79,80]. Thus, the accumulation of miR-706 in the nucleus would decrease its activity as a negative regulator of STAT1 in the cytoplasm, thus favouring myeloid differentiation. It has also been reported that miR-709 binds with perfect complementarity to pri-miR-15a and pri-miR-16-1 and inhibits the production of mature miRNAs [81]. Therefore, nuclear miRNAs can regulate the expression of other miRNAs through a novel interaction with their immature precursors. MiR-709 increases its expression and nuclear localization in granulocytes compared to promyelocytes. Several studies also show that miR-709 post-transcriptionally represses c-myc, whose expression levels are downregulated during myeloid cell differentiation [82]. Overall, the analysis of miR-690, miR-706 and miR-709 reveals new important mechanisms of functioning in the process of granulopoiesis. A first mechanism is the interaction and blockade at the nuclear level of the primary transcripts of other miRNAs. This results in the repression of the miRNAs and increased expression of their target genes. The other rather novel and peculiar mechanism is that miRNAs can be retained in the nucleus to decrease their cytoplasmic levels. As a consequence, the expression of its cytoplasmic target genes is increased [82].

7. Non Transcriptional Activities of Nuclear miRNAs

Nuclear miRNAs are reported to exert posttranscriptional regulation of RNA. Several data show the interaction between miRNAs and mRNA in the nucleus, where miRNAs recognise and bind to miRNAs responsive elements (MRE) sequences in the 3' or 5' UTR of the mRNA [83]. This interaction leads to the degradation of the mRNA, often involving AGO2 [84], but the mechanism of this effect has yet to be defined. MiRNAs can also target long non-coding RNAs (lncRNAs) within the nucleus. For example, miR-210 is reported to interact with a specific sequence present on XIST [84], a long non-coding RNA involved in mammalian X-chromosome inactivation during early female embryogenesis. XIST is also associated with cancer. In particular, its inappropriate expression/localisation on active X-chromosome is associated with breast cancer where miR-210 is highly expressed [85–88], further suggesting a regulatory activity of this miRNA on XIST. MiRNAs also locate within the nucleolus, where they may exert their activity by regulating the pri-miRNA levels. In particular, miR-122 localises in the nucleolus and binds to specific sequences on pri-miR-21. This interaction suppresses the miR-21 maturation induced by the Drosha complex and DGCR8 [89]. Other nucleolar miRNAs have been shown to interact with rRNAs. An example is miR-92a-2-3p, which recognises and binds a site on the 28S rRNA [90]. Other authors show an effect on the biogenesis of rRNAs, since the suppression of a minimal RISC formed by AGO2 and miRNAs leads to an increase in the amount of ribosomal RNA, suggesting a repressor role of miRNAs on rRNAs [91,92]. Lastly, it has been observed that miRNAs interact with their target mRNA already in the nucleolus. Here, they exert an initial pre-repression on the mRNA that will be completed in the cytoplasm [93]. In conclusion, the data suggest that nuclear miRNAs may act in part by regulating RNA processing.

8. Conclusion and Future Perspectives

It is well established that miRNAs play a critical role in many biological processes such as cell differentiation, lineage specification, cell cycle and immune response. In the common view, the activity of miRNAs is mostly restricted to the cytoplasm, where they regulate mRNA stability and translation. Our review highlights current evidence showing that miRNAs possess alternative mechanisms of action, that involve a nuclear activity. It is now clear that miRNAs localise in the nucleus, where they regulate gene expression through complex molecular mechanisms, including the regulation of transcription. MiRNAs can promote both activation and inhibition of transcription of their target genes, which are defined by miRNA-complementary sequences in their genomic loci. Thus, alteration of the miRNA biogenesis mechanism and miRNA expression levels may influence biological processes at multiple levels, including gene transcription. Indeed, the complexity of chromatin remodelers and miRNA-associated machineries contribute to dynamically modify the modality of genomic interaction, according to cell type, cell differentiation programs and cell metabolic status, requiring further focused studies. This generates a wide range of pathological

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consequences, including cell degeneration and neoplastic transformation. In this context, the activity of nuclear miRNAs needs further investigation in both physiological and pathological settings, especially in the definition of their role in tumorigenesis.

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