Minireview

Complexity in regulating microRNA biogenesis in cancer

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Impact statement

The canonical maturation pathway of miRNAs is highly conserved, indicating the crucial roles of these mini-regulators in most cellular processes. Dysregulation of specific miRNAs or imbalance of miRNA abundance has been observed in cancers. Accumulating evidence has shown that the interplay between miRNA processing factors and regulatory proteins previously known as key players in cancer malignancy regulates the biogenesis of miRNAs, expression of target genes, and eventually the alteration of cellular phenotypes. This minireview summarizes the current findings in the modulation of miRNA biogenesis in cancer to advance the understanding of how noncoding RNA contributes to cancer development and malignancy.

Abstract

The discovery of microRNA (miRNA) significantly extends our knowledge on gene regulation and noncoding gene functions. MiRNAs are important post-transcriptional regulators involve in a wide range of biological functions and diseases, including cancer. MiRNAs are produced by a unique biogenesis pathway involving the two-step sequential nuclear and cytoplasmic RNase-dependent processing at post-transcriptional level. However, a specific (set) of miRNA(s) is (are) synthesized under certain circumstance or developmental/pathological stage to fine-tune the gene expression profile. In this minireview, we will discuss the mechanism of miRNA biogenesis in cancer, mainly focusing on how Drosha and Dicer, two critical molecules controlling miRNA biogenesis, are modulated and which factor contributes to the specificity of selected miRNA maturation.

Keywords: Drosha, Dicer, miRNA biogenesis

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Introduction

The birth of miRNAs begins from the transcription of primary miRNAs (pri-miRNAs) or transcripts containing pri-miRNAs (e.g. intronic miRNAs) followed by two RNase-dependent steps mediated by nuclear Drosha/DGCR8 complex (microprocessor) and cytoplasmic Dicer complex to generate the end products, mature miRNAs. First, microprocessor removes the flanking regions of pri-miRNAs to generate shorter (~70 nt) miRNA precursors (pre-miRNAs). After transcription and nuclear processing, the pre-miRNAs are then recognized by Exportin-5 for nuclear-cytoplasmic transport. Subsequently, cytoplasmic pre-miRNAs are cleaved by Dicer complex, which contains Dicer, TARBP2 (HIV-1 TAR RNA binding protein), and PACT (protein activator of PKR) proteins.[1–4] As a molecular ruler, Dicer recognizes the basal end of pre-miRNA by its PAZ domain and determines the distance to cleavage site.[5] The following cleavage mediated by RNase III domains of Dicer removes terminal loop of pre-miRNA, leaving a double strand duplex of miRNA.[5,6] The miRNA duplex is then loaded into RNA-induced silencing complex (RISC) for unwinding and strand selection, which leaves the mature miRNA as a guide strand for sequence-specific target recognition and suppression (Figure 1).[7–9]

Abundance of miRNAs and expression of their biogenesis factors in cancer

Theoretically, the function of miRNAs is to mediate a sequence-specific target gene suppression. Thus, the role (oncogenic or tumor suppressive) of a given miRNA in cancer is supposedly dependent on its target(s) of

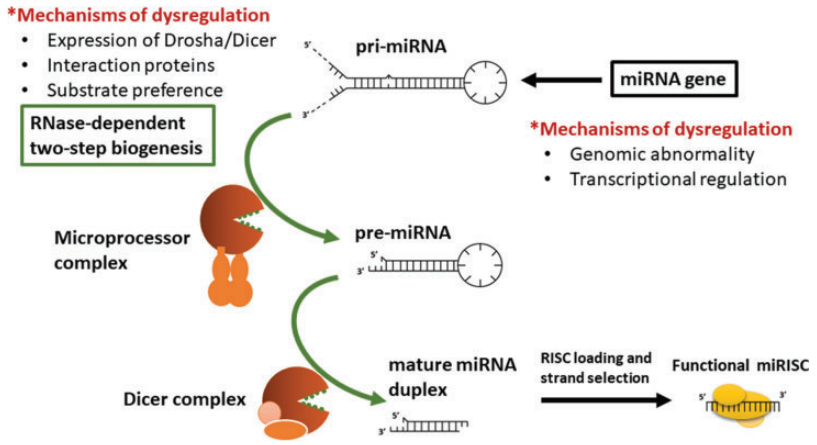

Figure 1. Canonical miRNA biogenesis pathway and the mechanisms of dysregulation. MiRNA is processed sequentially by the transcription of primary transcript (pri-miRNA) of miRNA gene or gene containing pri-miRNA sequence (intronic miRNAs), nuclear processing of pri-miRNA by microprocessor (Drosha/DGCR8) complex, and cytoplasmic maturation of pre-miRNA by Dicer complex. One of the strands of miRNA duplex is then loaded into AGO2-containing miRNA-induced silencing complex (RISC) forming functional miRISC to regulate sequence-specific target inhibition. Green arrows: RNase-dependent processing steps.

inhibition. MiRNAs suppressing oncogenes are thought as tumor suppressive miRNAs, while those targeting tumor suppressors are potentially oncogenic miRNAs (oncomirs).[10] Although different miRNAs may have independent roles in cancer and perform their tumor-promoting or -suppressing functions, it is intriguing that the expression of mature miRNA in tumor tissues is globally reduced in several cancer types,[11] implying that the biosynthesis of miRNAs may be inhibited in cancer. Indeed, a number of studies had reported the downregulation of Drosha and Dicer in some cancer types.[12] Paradoxically, several reports also suggested the upregulation of Drosha, Dicer, DGCR8, XPO5, and AGO2 in other cancers.[12] Thus, in addition to the possibility that these biogenesis factors may act through other miRNA-independent functions during tumorigenesis,[13] evidences show that the dysregulation of miRNA biosynthesis may be more complicated than we expected, owing to the complexity of genomic defects, upstream regulators, and auxiliary binding partners functioning at each maturation step. Interestingly, it is now getting clear that many protein factors involved in regulating cancer progression also contribute to managing miRNA processing and the resulting functional outcomes, demonstrating novel functions of these cancer-associated regulators in modulating miRNA expression. Next, we will highlight the major findings which define the ways of miRNA biogenesis dysregulation in cancer from genomic, transcriptional, to post-transcriptional levels.

Genetic abnormalities of DROSHA and DICER1 genes

Genetic abnormalities in genes encoding miRNA biogenesis factors were well-defined in Wilms tumor, a type of embryonal kidney neoplasia. Scientists identified somatic mutations in *DROSHA, DICER1*, *DGCR8*, *XPO5*, and *TARBP2* genes in Wilms tumors.[14–16] Interestingly, mutations in RNase domain-containing biogenesis factors *DROSHA* and *DICER1* were frequently detected in their RNAase IIIb domains. Moreover, high frequency of a recurrent mutation presented in metal-binding residue E1147K of RNAase IIIb domain was found in *DROSHA*-mutated tumors. Significant downregulation of a subset of mature miRNAs, not the primary miRNA transcripts, was further identified in *DROSHA*- and *DICER1*-mutated cells and tumors,[14,15] supporting their impacts in post-transcriptional control of miRNA processing. Gene editing and genetic manipulations in cancer cells indicated that the heterozygous E1147K-mutated *DROSHA* would function as dominant-negative rather than in a haploinsufficient manner.[15,17] In addition to Wilms tumor, mutations in *DICER1* were detected in pleuropulmonary blastoma (PPB),[18] cystic nephroma,[19] pituitary blastoma,[20] embryonal rhabdomyosarcoma (ERMS),[21] nonepithelial ovarian cancer,[22–24] and ovarian embryonal rhabdomyosarcoma.[25] With the notion of broader germline mutations observed in *DICER1*, it is supposed that these *DICER1* mutations are associated with inherited cancers. Causatively, loss of normal functions of Drosha and Dicer may lead to the reduced processing of miRNAs, which is consistent with the findings that miRNAs are globally downregulated in cancer. These reports indicate the importance and pathological relevance of the genomic integrity of miRNA biogenesis factors in cancer, especially those with inherited cancer syndromes.

Transcriptional regulation of Drosha and Dicer

Primary miRNA transcription is regulated by transcriptional regulators controlling the expression level of itself (from independent miRNA gene) or transcripts of its host gene (from genes containing intronic miRNAs). Thus, it is not surprising that the transcriptional regulation contributes to the spatial and temporal modulation of specific primary miRNA transcripts. Then, the regulatory mechanism of miRNA biogenesis, which is controlled by abovementioned protein factors, contributes to another layer of regulation at post-transcriptional level. Since these protein

factors are still derived from their own genes, they are also controlled by transcriptional regulation.

One example is ETS1/ELK1-mediated downregulation of Drosha in ovarian cancer.[26] There are binding sites for ETS1/ELK1 closely located to the transcription initiation site of *DROSHA*. Upon hypoxia, expression of *DROSHA* is transcriptionally suppressed when ETS1/ELK1 binds to its promoter regions, leading to the inhibition of miRNA biogenesis and consequently facilitating EMT, tumor growth, metastasis, and cancer progression.[26] Moreover, a parallel research demonstrated that H3K27me3 is enriched in *DICER1* promoter under hypoxia via the dynamically regulated epigenetic modification by EZH2 methyltransferase and the KDM6A/B demethylases.[27] The resulting defect of Dicer-mediated miRNA processing and reduced mature miR-200 family miRNAs promotes stem cell-like phenotype, EMT, and is associated with poor prognosis of breast cancer.[27] Su *et al.* showed that another transcription factor of p53 family member, Tap63, binds to *DICER1* promoter. This transcriptional activation promoted Dicer expression and maturation of miR-130b to suppress metastasis.[28] These studies highlight the transcriptional regulation directly modulating the expression of Drosha and Dicer in cancer. The central roles of miRNA biogenesis pathway are also demonstrated as the upstream factors (hypoxia, ETS1/ELK1, KDM6A/B, Tap63), downstream targets (miR-200, miR-130b), and the resulting phenotypic consequences (stem cell-like phenotype, EMT, metastasis) are well-defined key players in cancer progression.

Regulatory interaction proteins for Drosha and Dicer complexes

Since the post-transcriptional biosynthesis of miRNA is a stepwise maturation process that requires nuclear microprocessor and cytoplasmic Dicer complex to sequentially cleaved miRNA precursors, the expression and activities of the biogenesis factors/complexes determine the abundance of mature miRNAs. In addition to the transcriptional regulation of Drosha and Dicer discussed in previous section, accumulating evidence show a number of proteins are capable of interacting with the biogenesis factors or with specific miRNA precursors, resulting in the changes in complex activities and regulation of miRNA maturation, editing, and functions. These regulators are included but not limited to protein factors previously known as canonical DNA-binding transcription factors/regulators, RNA-binding proteins, and RNA-editing enzymes (Figure 2). The mode of actions and target miRNAs of these regulators is summarized as followings.

DNA-binding transcription factors/regulators

Canonically, proteins binding to DNA are generally considered to have genomic or genetic functions, such as transcription factors. These factors have long been known to participate in many biological pathways, whereas proteins having RNA-binding ability were independently considered as another group of nucleic acid-binding proteins with distinct functions. Now, this concept is outdated and has been redefined partly because of several major findings indicating that DNA-binding proteins are able to interact with miRNA precursors or RNA-binding proteins involved in miRNA processing.

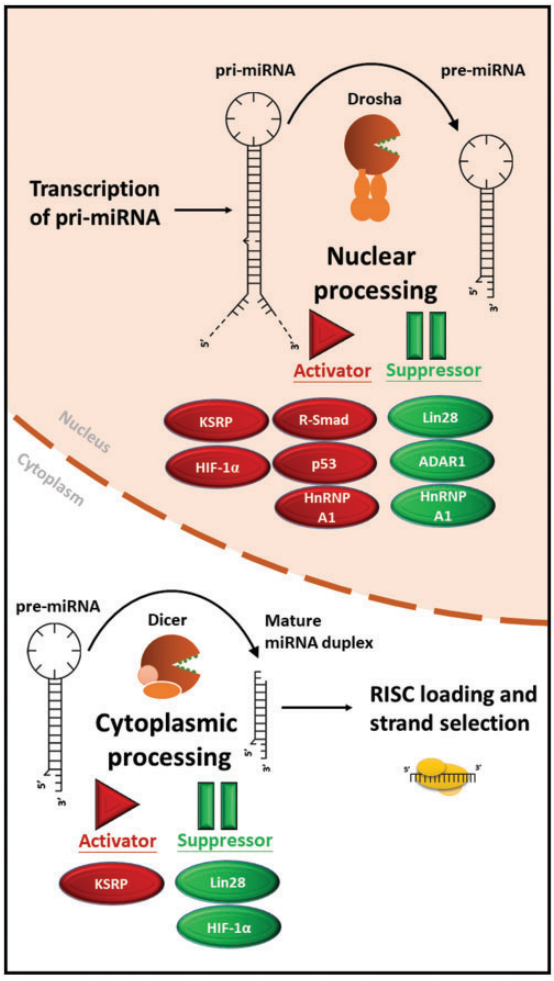

Figure 2. Regulatory interaction proteins modulate miRNA processing. Activators (red) and suppressors (green) involved in managing miRNA processing occurred at either nuclear or cytoplasmic step is illustrated.

Smads. Transforming growth factor beta (TGF-β) is a cytokine involving in the regulation of cancer cell growth, apoptosis, differentiation, and EMT.[29] Several studies indicated that TGF-β modulates the expression of different miRNAs both in transcriptional and post-transcriptional manners.[30] At post-transcriptional level, signaling of TGF-β induces its canonical downstream transcription factors, R-Smads, to interact with microprocessor-associated RNA helicase, DDX5 (also called p68), and facilitate pri-miR-21 processing.[31] In this case, a subset of pri-miRNAs are processed into pre-miRNAs by enhanced microprocessor activity and is accumulated in cells.[31] One of these miRNAs, miR-21, is a well-established oncogenic miRNA,[10] implying a mechanism of TGF-β/R-Smads-induced miRNA-specific regulation during tumorigenesis. Supportively, a later report also revealed the conserved sequence motif in TGF-β-regulated miRNAs. An R-Smads-binding element in pri-miR-21 was

identified and proved to be essential for TGF-β/R-Smads-induced pri-miR-21 cleavage, providing evidence for another layer of miRNA-specific regulation.[32]

P53. P53 is a well-known nuclear transcription factor which contributes to tumor suppression.[33] In 2009, another similar mechanism of p53-regulated miRNA biogenesis was identified. P53 is a well-known tumor suppressor transcriptionally regulating a number of tumor suppressors as well as several tumor suppressive miRNAs such as miR-34.[34–36] Like R-Smads, the non-canonical function of p53 is to promote microprocessor activity through the interaction with DDX5 and subsequently facilitate the nuclear processing of pri-miR-16-1, -143, -145, and-206.[37] These miRNAs were later found as potential tumor suppressors that targeted K-Ras and CDK6.[37] Similar to the canonical pathway of p53, this non-canonical mechanism is also triggered by DNA damage. Interestingly, Chang *et al.* further reported that the acetylation of p53 at K120 is induced by genotoxic stresses. Without affecting the transcriptional activity of p53, the association of acetylated p53 with microprocessor complex was enhanced to process the apoptosis-promoting miR-203 maturation.[38]

HIF-1α. Hypoxia-inducible factor-1-alpha (HIF-1α) is a transcription factor in response to oxygen level of microenvironment. Under tumor hypoxia, HIF-1α is stabilized and transactivates downstream genes to promote genes for hypoxia adaption and tumor progression.[39–41] It was reported that HIF-1α interacts with Drosha to facilitate pri-miR-215 incorporation into microprocessor, resulting in enhanced pri-miR-215 maturation and expression of mature miR-215.[42] As a result, miR-215 directly inhibited the histone demethylase KDM1B expression and contributed to adaptation to hypoxia in glioma-initiating cells.[42] In addition, HIF-1α also interacts with Dicer and promotes its Parkin-dependent ubiquitination and following autophagic degradation.[43] Degradation of Dicer hindered miR-200b maturation and thus unleashed the expression of ZEB1 to promote EMT and cancer metastasis.[43]

RNA-binding proteins

The RNA-binding ability is required for dsRBD domain-containing Drosha, DGCR8, and Dicer to properly interact with miRNA precursors. In addition to these canonical RBPs involved in miRNA biogenesis, a growing body of evidence have shown that other factors previously known to have RNA-binding ability unexpectedly regulate miRNA processing through the association with microprocessor, Dicer complex, or specific miRNA precursors. Although they regulate miRNA processing through different ways, the fundamental RNA-binding abilities provide them to recognize and modulate the maturation of specific substrates (pri- or pre-miRNAs). Through interacting with Drosha or Dicer complex, these RBPs greatly extend the possibility of individual miRNA regulation.

KSRP. KH-type splicing regulatory protein (KSRP) is a RBP recognizing mRNA containing AU-rich destabilizing elements and directing these elements to RNA degradation.[44,45] Trabucchi *et al.*[46] showed that KSRP also associates with either microprocessor or Dicer complex. As a co-activator of the processing complexes, KSRP interacts with the terminal loops of a subset of pri-or pre-miRNAs to facilitate their maturation, following by targeting mRNAs inhibition and modulations on cell proliferation, differentiation, and apoptosis.[46] The KSRP-regulated miRNA biogenesis was later revealed to be enhanced by DNA damage, resulting from ATM-mediated KSRP phosphorylation and activation.[47] Ubiquitination of KSRP at K87 induced by hypoxia was reported to suppress its interaction with pri-miRNAs and microprocessor and therefore inactivating nuclear processing of let-7 and promoting tumorigenic activity.[48]

HnRNPA1. Another RBP, heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1), has also been reported to bind to specific miRNA precursors and modulate their maturation. The first study published in 2007 showed that hnRNP A1 interacts with pri-miR-18a before microprocessor was processed and subsequently enhances miR-18a maturation and function in target suppression.[49] One of the strand of mature miR-18a duplex, miR-18a-3p, was later shown to potentially function as a tumor suppressor by targeting K-Ras.[50] In contrast to promoting miR-18a maturation, it was reported that hnRNP A1 exhibits an antagonistic function along with KSRP on the binding and processing of let-7 precursors and later on causing the decreased maturation of let-7a.[51] Since let-7a is a well-known tumor suppressive miRNA targeting K-Ras,[52,53] the effect of hnRNP A1 on inhibiting let-7 suggested its oncogenic role, which is in contrast to its potential tumor suppressive effect on upregulating miR-18a-3p and the resulting K-Ras inhibition.[50,51] In pri-miR-18a highly expressed cells, the way to enhanced processing and expression of mature miR-18a-3p by hnRNP A1 is potentially tumor suppressive, while hnRNP A1-suppressed let-7 may have oncogenic effect. Thus, the functional role of hnRNP A1 in cancer may be determined by another layer of regulation. For example, relative abundance of steady-state level of pri-miR-18a or pri-let-7a may determine what miRNA will be processed by hnRNP A1-mediated maturation or not.

Lin28. Lin28 is a highly conserved RBP involved in development and oncogenesis in multiple cancer types.[54] During 2008–2009, a number of studies reported the Lin28-mediated uridylation of pre-let-7.[55,56] Pre-let-7 with uridylated 3′UTR undergoes RNA decay and is failed to be processed by Dicer.[55] The TUTase4 (TUT4, also called Zcchc11) was further revealed as the uridylyl transferase specifically recognized a tetra-nucleotide sequence motif in terminal loop of pre-let-7 precursors and then added an oligouridine tail to the 3′UTR of pre-let-7.[56,57] Similarly, a subset of additional miRNAs with the same sequence motifs in their terminal loops were also identified to be regulated by Lin28/TUT4-mediated uridylation.[56] Viswanathan *et al.* showed that Lin28 protein also

suppresses microprocessor-dependent pri-let-7 processing.[57] Lin28A and Lin28B are two Lin28 gene in human sharing high homology. Unlike the Lin28A/TUT4-mediated uridylation on pre-let-7, Piskounova *et al.*[58] and Newman *et al.*[59] uncover that Lin28B blocks pri-let-7 processing in a TUT4-independent fashion. As a regulatory feedback between Lin28 and let-7, Rybak *et al.*[60] found that let-7 is a negative regulator of Lin28 during neural stem-cell commitment. Together with these evidence, the Lin28/let-7 pathway is a fine-tuned circuit involving multiple crosstalk mechanism, which link the oncogenic Lin28 and tumor suppressive let-7 in cancer. Since PD-L1 was recently identified as a target of let-7, the Lin28/let-7 pathway was reported to be involved in the regulation of immune evasion of cancer cells.[61]

ADAR1. The adenosine deaminases acting on RNA (ADARs) are RBPs catalyzing the A-to-I nucleotide editing of RNA at post-transcriptional level.[62] Nemlich *et al.*[63] found that loss of ADAR1 enhanced tumorigenesis is during metastatic transition of melanoma. They further identified a number of miRNAs are regulated by ADAR1 dependent on its RNA-binding ability. Mechanistically, ADAR1 occupied DGCR8 from microprocessor formation to inhibit nuclear processing of pri-miRNAs, and indirectly downregulated Dicer expression through upregulation of let-7.[63] In addition to the mechanism relies on RNA-binding ability of ADAR1, other studies reported the ADARs-mediated miRNA editing and the functional changes based on the alterations of preference for their target selection,[62,64] which is not discussed here.

In summary, since many of the studies demonstrated the selective binding of these factors to pri- or pre-miRNAs which lead to the dysregulated maturation of given miRNA(s), the machinery controlling specific miRNA processing beyond abovementioned global miRNA dysregulation provides another layer of mechanism for precisely management of a subset of miRNAs to regulate cellular functions which is still under investigation.

Conclusion

Dysregulation of miRNA, either in a global reduction or a subset of altered miRNA expression, is a widespread phenomenon in cancer. Functional chaos of these miRNAs could be the results from the abnormal birth, maturation, turnover, and the editing of mature miRNAs and their precursor. In this minireview, we summarize the regulatory mechanisms of miRNA maturation at different molecular levels, including genetic abnormality, epigenetic silencing, to post-transcriptional modulations on regulating processing activity in cancer. These researches extend our knowledge on the complexity of miRNA processing and the crosstalk to oncogenic or tumor suppressive signaling network. Noteworthy, a significant number of these regulators interacting with microprocessor, Dicer complex, or miRNA precursors are protein factors originally known to have DNA- or RNA-binding domain, which integrates the new functions of these nucleic acid-binding proteins in miRNA biogenesis, and may provide a way back from miRNA to interfere the known functions of these regulators. Since miRNAs require their interplay with protein factors to be processed or to form functional complex, crosstalk between canonical miRNA biogenesis factors and the newly-identified regulatory proteins orchestrate the cancer-specific regulation of miRNA biogenesis.

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