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Interplay between miRNAs and host genes and their role in cancer

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Abstract

MicroRNAs (miRNAs) are small endogenous non-coding functional RNAs that post-transcriptionally regulate gene
expression. They play essential roles in nearly all biological processes including cell development and differentiation, DNA
damage repair, cell death as well as intercellular communication. They are highly involved in cancer, acting as tumor
suppressors and/or promoters to modulate cell proliferation, epithelial-mesenchymal transition and tumor invasion and
metastasis. Recent studies have shown that more than half of miRNAs are located within protein-coding or non-coding
genes. Intragenic miRNAs and their host genes either share the promoter or have independent transcription. Meanwhile,
miRNAs work as partners or antagonists of their host genes by fine-tuning their target genes functionally associated with
host genes. This review outlined the complicated relationship between intragenic miRNAs and host genes. Focusing on
miRNAs known as oncogenes or tumor suppressors in specific cancer types, it studied co-expression relationships between
these miRNAs and host genes in the cancer types using TCGA data sets, which validated previous findings and revealed
common, tumor-specific and even subtype-specific patterns. These observations will help understand the function of
intragenic miRNAs and further develop miRNA therapeutics in cancer.

Key words: miRNA; intragenic miRNA biogenesis; host genes; interplay; cancer

Introduction

MicroRNA (miRNA) is a class of small endogenous non-coding
RNAs with a length between 20 and 24 nucleotides. They recog-
nize and bind mRNA transcripts in a sequence-specific mode,
resulting in degradation or translational repression of the corre-
sponding mRNAs [1, 2]. The generation of miRNA from primary
miRNA is a multi-step but fairly well understood process. After
cleavage of primary transcripts to form precursor miRNAs in the
nucleus, precursor miRNAs are exported into the cytoplasm for
additional cleavage by an enzyme called Dicer to produce single-
stranded mature miRNAs. Mature miRNAs are then loaded onto
the RNA-induced silencing complex where they bind to 3′-UTR
sequences of target genes. If the 3′-UTR of the target mRNA
is fully complementary to the miRNA seed site, the mRNA is
targeted for degradation. If it is partially complementary to
the miRNA, the mRNA is targeted for translational inhibition
[3–5]. In this way, an individual miRNA can post-transcriptionally

regulate expression of hundreds of mRNAs, which profoundly alter gene expression and drive cells toward transformation.

Cancer is a complex disease involving multiple genes and pathways; therefore, it is not very useful to target one single gene for cancer treatment. A single miRNA can regulate expression of hundreds of genes involved in distinct cellular functions, which makes miRNAs the promising therapeutic targets. Since the miRNA dysregulation in cancer was first reported in 2002 [6], many studies have found miRNAs' function in carcinogenesis including tumor suppressor miRNAs (ts-miRs) and oncogenic miRNAs (onco-miRs). The upregulated onco-miRs and downregulated ts-miRs lead to cancer initiation and progression [7, 8]. These miRNAs have attracted lots of attention as biomarkers and therapeutic targets. Controlling miRNAs transcriptional regulation by genome or RNA-editing tool has become a novel and promising approach [9, 10].

Although there is a general understanding of the generation of mature miRNA from primary miRNA, the transcriptional regulation of primary miRNAs is still unclear. The location of miRNAs is one major factor that determines how primary miRNAs are transcriptionally regulated. According to their genomic position, miRNAs can be classified into intragenic and intergenic miRNAs. Intragenic miRNAs, embedded within introns or exons of genes on the same strand, are believed to be co-regulated with their host genes by Pol II, whereas intergenic miRNAs, located between genes, are transcribed from their own Pol II or Pol III promoters. Recent studies, however, revealed that intragenic miRNAs, even intronic miRNAs, are not always co-transcribed with their host genes [11, 12]. Some intronic miRNAs have been found to interact with their host genes to affect their stability [13, 14]. More importantly, bioinformatics studies predicted that approximately 20% of intronic miRNAs target host mRNA transcripts in a feedback loop [13–15].

In this review, we outlined the complicated relationships between miRNAs and their host genes. Focusing on onco-miRs and ts-miRs in specific cancer types, we studied co-expression relationships between these miRNAs and host genes using TCGA data sets. We not only found common co-expression patterns, but also revealed tumor-specific and even subtype-specific relationships, which are very helpful for understanding transcriptional and post-transcriptional regulation of miRNAs and further developing miRNA therapeutics in cancer.

Definition of intragenic miRNAs and their host genes

Location of miRNAs is one of the hot topics in miRNA research since it is a major factor to determine how miRNAs are regulated. Based on their location, miRNAs can be classified into intragenic miRNAs and intergenic miRNAs. miRNAs located within protein-coding or non-coding genes are called intragenic miRNAs, while the genes in which the miRNAs are embedded are called host genes. Intragenic miRNAs can be further categorized into intronic miRNAs (miRNAs located in intronic regions), exonic miRNAs (miRNAs located in exonic regions), junction miRNAs (miRNAs located in gene intron–exon junctions) and antisense miRNAs (miRNAs located in the antisense strand of gene) (Figure 1). Intergenic miRNAs, located between genes, are believed to be transcribed from their own Pol II or Pol III promoters. However, recent studies revealed that there is a small percentage of intergenic miRNAs that are potentially co-transcribed with their neighboring genes through two mechanisms. One mechanism is called readthrough, where the transcription of one gene continues beyond the normal transcription termination site into intergenic regions. miRNAs located in the immediate
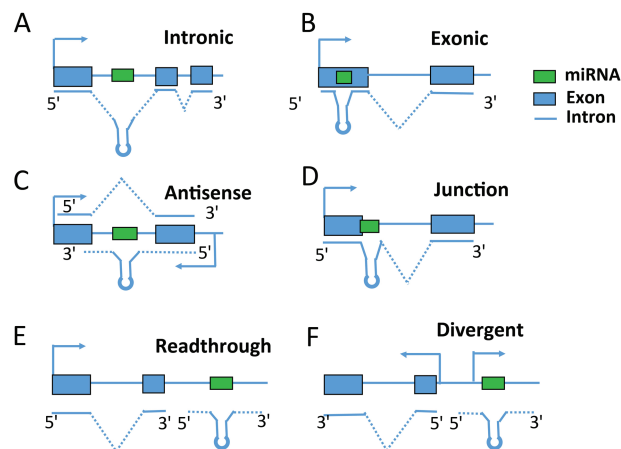

Figure 1. Intragenic miRNA classification. miRNA categories are defined based on their locations relative to exon, intron and gene regions.
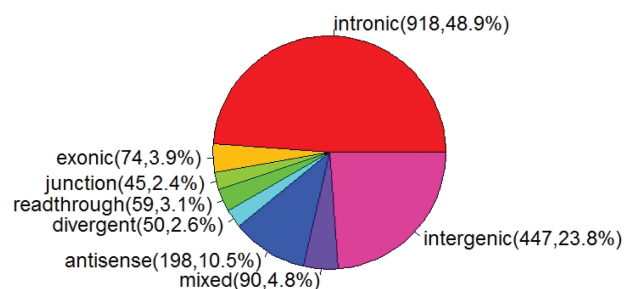

Figure 2. Pie chart of miRNA categories.

(<4000 bp) downstream sense region of protein-coding or non-coding genes are likely to be transcribed by readthrough transcription [16] (Figure 1E). The other is called divergent transcription, defined as two polymerases transcribing on both sense and antisense directions from the same promoter. As a common feature for active promoters, divergent transcription generates long non-coding antisense transcripts coupled to active gene promoters [16–18] (Figure 1F). These two types of intergenic miRNAs (readthrough or divergent) can be classified into 'intragenic' category as well since they potentially share the same promoter with their neighboring genes, which are different from other intergenic miRNAs in transcriptional regulation. The neighboring genes that miRNAs are potentially co-transcribed with can also be called 'host' genes.

Previous studies have reported that more than half of miRNAs are intragenic miRNAs. To facilitate the analysis of genomic and structure features of intragenic miRNAs and host genes, miRIAD (http://www.miriad-database.org) provides a comprehensive knowledge on intragenic miRNAs by collecting public data from multiple sources, such as host gene function, miRNA and host gene expression and miRNA targets [19]. As gene annotation is improved and new miRNAs are identified, more intragenic miRNAs are classified [20]. To date, there are 1881 miRNAs in miRBase v21 [21–25]. Based on their relative location to protein-coding or non-coding genes (Figure 1), 1434 can be classified as intragenic miRNAs, among which 918 (48.8%), 74 (3.9%), 45 (2.4%), 59 (3.1%), 50 (2.6%), 198 (10.5%) are intronic, exonic, junction, readthrough, divergent and antisense miRNAs, respectively (Figure 2). There are 90 miRNAs (4.8%), which can not be assigned into one specific category. For example, an miRNA is located within the intron region of one transcript and also

embedded in exon regions of another transcript. In this case, this miRNA is assigned into a 'mixed' category (Figure 2).

Interplay between intragenic miRNAs and their host genes

Early evidence proposed a co-expression model suggesting that intronic miRNAs are derived from the same primary transcripts as their host genes. Intronic miRNAs use the transcriptional start sites (TSSs) of their host genes to initiate transcription. The primary transcript is then processed into mRNA and miRNAs by microprocessor and splicing. There is no interplay between microprocessor cleavage and splicing, resulting in the co-expression between miRNA and mRNA [26–28]. For example, the expression levels of miR-335 significantly correlated with *MEST* in 21 HCC cell lines, supporting the notion that the intronic miRNA is co-expressed with its host gene under the regulation of the host gene promoter [29]. Strongly correlated expression profiles between 175 miRNAs and their host genes across 24 different human organs have been reported [26]. An extreme case of coordination between intronic miRNA processing and splicing happens in very short introns, where the entire intron is so short that it is already a pre-miRNA without the involvement of microprocessor. These short introns, called mirtrons, are highly co-expressed with their host genes [30].

Increasing evidence, however, has shown that intronic miRNAs and host genes are not co-expressed as frequently as we previously expected due to two main reasons [31, 32]. One reason is that miRNAs have their own independent promoters. It has been found that over one-third of intronic miRNAs have their own promoters (Polymerase II or III), whose transcription is independent from their host genes [11, 33]. For example, it has been validated that miR-26b has an independent TSS, promoter signal and transcriptional factor from its host gene *CTDSP* [31]. Kim *et al.* [34] revealed that the splicing factor SRSF3 regulates the expression of miR-1908 independently from its host gene *FADS1*, suggesting that miR-1908 could be affected by its own transcription unit. Ozsolak *et al.* [35] reported that roughly 30% of intragenic miRNAs may be initiated independently especially if they are resided in large-sized (>5 kbp) intronic segments. The intronic regions are long enough to carry their own transcription units of pre-miRNA genes [32]. The other reason is the crosstalk between microprocessor cleavage and splicing [36]. In addition, the evolution conservation also contributes to the expression discordance between miRNAs and their hosts. He *et al.* [11] reported that evolutionary non-conserved intragenic miRNAs are less co-expressed with host genes than conserved ones. They suggest that most non-conserved miRNAs are expressed serendipitously and weakly but not necessarily always from the same transcripts as the host genes and some are co-expressed owing to shared chromatin dynamics. They then conjecture that for some of these their co-expression is beneficial and selection favors them to be what call 'embedded', meaning they evolve stronger co-expression, which in turn favors conservation of miRNA target sites on 3′-UTRs of desirable target genes [11].

The transcriptional regulation of exonic miRNAs is different from intronic miRNAs since Drosha processing of an exonic miRNA will inhibit production of the spliced host mRNA [37, 38]. Sundaram *et al.* [37] showed a clear inverse correlation between the expression pattern of miR-198 and *FSTL1* (host gene), which highlights the importance of this regulatory switch in controlling context-specific gene expression to orchestrate wound re-epithelialization.

The biogenesis of junction miRNAs is most complicated since there is competition between spliceosome and the microprocessor. When the splicing machinery does not recognize the internal exon, and the microprocessor components, rather than the spliceosome complex, bind to the RNA transcript at the pre-miRNA region to generate pre-miRNA. When the internal exon is recognized through interaction with specific splicing factors, the RNA secondary structure is released and splicing of the internal exon is carried out by the spliceosome before DGCR8 and Drosha bind to the pre-miRNA region. The spliced variant containing the alternative exon is the product and no pre-miRNA is made [39]. That is to say, junction miRNA is negatively regulated by splicing. Melamed *et al.* [39] studied an miRNA cluster including one exonic miRNA miR-410, one intronic miRNA miR-541 and one junction miRNA miR-412. They confirmed that the splicing and the processing of pre-miRNAs located in exon-intron junctions are mutually exclusive and alternative splicing is inversely correlated with levels of miR-412.

Readthrough and divergent miRNAs are special whose transcription is a byproduct of their neighboring genes. Readthrough miRNA is generated by a continuous transcription beyond a normal stop sign from an active gene (Figure 1E). For example, miR-21 is the most commonly over-expressed miRNA in cancer and a proven oncogene. miR-21 is located immediately downstream of the vacuole membrane protein-1 (*VMP1*) gene, which was reported to bypass the polyadenylation signals to include miR-21, thus providing a novel and independently regulated source of miR-21, termed VMP1–miR-21 [38]. Divergent miRNA is a product of bi-directional transcription of active promoters. In humans, some bidirectional promoter regions transcribing an miRNA and protein-coding gene simultaneously have been experimentally identified, e.g. the BTG4 promoter upstream of miR-34b and miR-34c [40], and the *POLR3D* promoter upstream of miR-320a on chromosome 8 [41].

In addition to the transcriptional regulation of intragenic miRNAs by their host genes, host genes are also subject to the regulation of miRNAs. The complicated and mutual regulation between miRNAs and host genes form a negative or positive feedback loop, where miRNAs play an antagonistic or synergetic role as a competitor or partner of a host gene [13, 14]. Bioinformatics studies have demonstrated that approximately 20% of intronic miRNAs were predicted to target their host genes [13]. One example is miR-338-3p, which inhibits the transcription of its host gene *AATK* by binding to its 3′-UTR [42]. Another example is miR-26b, which controls neuronal differentiation by repressing its host transcript, *ctdsp2* [43]. Such an interaction forms the 'first-order' negative-feedback circuit for fine-turning host gene expression. Besides direct regulation, an miRNA can negatively regulate its host gene function by targeting a gene whose product is a downstream effector of the host gene product. Such regulation could be termed 'second-order' negative feedback. For example, miR-641 is not predicted to target its host, *AKT2*, while its predicted targets, which include *PI3K*, *EGFR*, *PTEN*, *PDK1*, *RAS*, *MEK* and *ERK*, are in functional synergy with its host, giving credence to the second-order negative-feedback circuitry line of evidence [44].

Association between intragenic onco/ts-miRNAs and their host genes in cancer

Disruption of miRNA expression is frequently observed in cancer. They can act as oncogenes and/or tumor suppressors. Study of a large collection of chronic lymphocytic leukemias (CLLs) showed knockdown or knock out of miR-15a and miR-16-1

Table 1. Summary of co-expression patterns between onco/ts-miRs and their host genes in specific cancer types

miR cluster	miRNA	Host	Loc	Type	Cancer	Correlation in TCGA	Ref.
miR-1	miR-1-1	MIR1-1HG	Intron	ts-miR	Thyroid; bladder cancer	–	[52, 53]
miR-9	miR-9-1	C1orf61	Intron	onco-miR	Breast cancer	0.39**	[54–56]
	miR-9-3	MIR9-3HG	Mix	ts-miR	Gastric cancer	0.25**	[56]
miR-15/16–1	miR-15a	DLEU2	Intron	ts-miR	CLL	–	[57, 58]
	miR-16-1					–	
miR-17-92	miR-17	C13ORF25	Mix	onco-miR	Lymphomas; lung cancer; renal cell cancer	–, 0.49**, 0.07	[57–59]
	miR-18a					–, 0.48**, 0.01	
	miR-19a					–, 0.50**, 0.11	
	miR-20a					–, 0.55**, 0.07	
	miR-19b1					–, 0.48**, 0.16	
	miR-92a1					–, 0.51**, 0.19	
miR-21	miR-21	VMP1	Read through	onco-miR	Breast cancer	0.52**	[60–65]
					Lung cancer	0.36*	
					Pancreas cancer	0.61**	
					Liver cancer	0.40	
					Gastric cancer	0.54**	
					Cervical cancer	0.39**	
					Hematopoietic cancer	–	
miR-23b	miR-23b	C9ORF3	Intron	ts-miR	Gastric cancer	0.65**	[66]
miR-26a	miR-26a-1	CTDSPL	Intron	ts-miR	Osteosarcoma; colorectal cancer	–,–	[67–69]
	miR-26a-2	CTDSP2				–,–	
miR-26b	miR-26b	CTDSP1	Intron	ts-miR	Colorectal cancer	–	[67, 69]
miR-27b	miR-27b	C9ORF3	Intron	ts-miR	Prostate cancer	0.62**	[60, 70]
miR-30c	miR-30c-1	NFYC	Intron	ts-miR	Prostate cancer	0.06	[71]
miR-33a	miR-33a	SREBF2	Intron	ts-miR	Prostate cancer	0.24**	[72]
miR-34b	miR-34b	BTG4	Divergent	ts-miR	Breast cancer	−0.05	[73]
					Prostate cancer	–	
miR-95	miR-95	ABLIM2	Intron	onco-miR	Liver cancer	0.75**	[74]
miR-101	miR-101-2	RCL1	Intron	ts-miR	Gastric cancer	0.17*	[75]
miR-106b/25	miR-106b	MCM7	Intron	onco-miR	Breast cancer	0.68**	[76]
	miR-93					0.57**	
	miR-25					0.66**	
miR-107	miR-107	PANK1	Intron	ts-miR	Pancreatic cancer	0.22*	[77]
miR-125b	miR-125b-1	MIR100HG	Intron	onco-miR	Lung cancer	0.69**	[63]
	miR-125b-2	MIR99AHG				0.77**	
miR-126	miR-126	EGFL7	Intron	ts-miR	Pancreatic cancer	0.56**	[78]
					Breast cancer	0.13*	
					Lung cancer	0.28	
miR-128	miR-128-1	R3HDM1	Intron	ts-miR	AML	–	[79–81]
					Esophageal cancer	–	
					Glioblastoma	–	
miR-137	miR-137	MIR137HG	Exon	ts-miR	Neuroblastoma	–	[82, 83]
miR-139	miR-139	PDE2A	Intron	ts-miR	Endometrial; colorectal cancer	–	[67, 84, 85]
miR-149	miR-149	GPC1	Intron	ts-miR	Breast cancer	0.37**	[86]
miR-152	miR-152	COPZ2	Intron	ts-miR	Prostate cancer	0.31**	[67]
					Endometrial cancer	0.70**	
miR-153	miR-153-1	PTPRN	Intron	ts-miR	Ovarian cancer	–	[87]
					Glioblastoma	–	
					Gastric cancer	−0.007	
	miR-153-2	PTPRN2			Ovarian cancer	–	[87]
					Glioblastoma	–	
					Gastric cancer	0.45**	
miR-155	miR-155	MIR155HG	Exon	onco-miR	B-cell cancers	–	[88–91]
					Glioblastoma	0.68**	
miR-181a	miR-181a-1	MIR181A1HG	Intron	onco-miR	Cervical cancer	–	[92, 93]
					Breast cancer	–	
	miR-181a-2	MIR181A2HG			Cervical cancer	–	
					Breast cancer	–	
miR-185	miR-185	TANGO2	Intron	ts-miR	Colorectal cancer	–	[94]
					Lung cancer	0.49**	
					Breast cancer	0.36**	

(Continued)

Table 1. *(Continued)*

miR cluster	miRNA	Host	Loc	Type	Cancer	Correlation in TCGA	Ref.
miR-186	miR-186	ARANB2	Intron	ts-miR	Glioblastoma	0.4**	[95]
miR-198	miR-198	FSTL1	Exon	ts-miR	Breast cancer	−0.04	[96]
miR-210	miR-210	MIR210HG	Intron	onco-miR	Pancreatic cancer	–	[97]
miR-218	miR-218-1	SLIT2	Intron	ts-miR	Breast cancer	0.17**	[98]
					Gastric cancer	0.66**	
	miR-218-2	SLIT3			Breast cancer	0.33**	118
miR-224/452	miR-224	GABRE	Intron	onco-miR	Gastric cancer; lung cancer	0.69**, 0.83**	[99]
						0.75**, 0.80**	
	miR-452			ts-miR	Prostate cancer	0.58**, 0.63**	
miR-326	miR-326	ARRB1	Intron	ts-miR	Lung cancer	0.41**	[67, 100]
miR-335	miR-335	MEST	Intron	onco-miR	Ovarian cancer	0.66**	[101, 102]
				ts-miR	Gastric cancer	0.79**	[103, 104]
					Liver cancer	0.88**	
					Breast cancer	0.78**	
miR-338	miR-338	AATK	Intron	ts-miR	Breast cancer	0.61**	[105]
miR-340	miR-340	RNF130	Intron	onco-miR	Gastric cancer	0.35**	[99]
				ts-miR	Breast cancer	0.28**	[106]
miR-342	miR-342	EVL	Intron	ts-miR	Colorectal cancer	–	[107]
miR-346	miR-346	GRID1	Intron	ts-miR	Liver cancer	0.10	[108]
miR-449a/b	miR-449a	CDC20B	Intron	ts-miR	Neuroblastoma	0.23**, 0.61**	[109]
	miR-449b				Prostate cancer	0.09, −0.25**	[110]
miR-486	miR-486	ANK1	Intron	ts-miR	Esophageal cancer	0.31**	[111]
miR-488	miR-488	ASTN1	Intron	ts-miR	Gastric cancer	−0.2*	[112]
miR-489	miR-489	CALCR	Intron	ts-miR	Esophageal cancer	0.02	[113]
miR-491	miR-491	FOCAD	Intron	ts-miR	Colorectal cancer	–	[114]
miR-504	miR-504	FGF13	Intron	onco-miR	HSCC	–	[115–117]
					Neuroblastoma	–	
				ts-miR	Glioblastoma	0.67**	
miR-634	miR-634	PRKCA	Intron	ts-miR	Breast cancer	–	[118]
miR-1908	miR-1908	FADS1	Intron	onco-miR	Glioblastoma	−0.12*	[57, 58]
					Ovarian cancer	0.01	

Cells with '–' means correlation coefficients were not available since the expression of miRNA and/or its host gene were undetected in this cancer type.

*Represents the FDR ≤ 0.01.

**Represents the FDR ≤ 0.001.

in approximately 69% of CLLs. This is the first evidence that miRNAs might be involved in the pathogenesis of human cancer [6]. Frixa *et al.* [45] reported that miR-128-3p, upregulated in lung cancer, targets Drosha and Dicer, two key enzymes of miRNAs processing, leading to the widespread down-regulation of miRNA expression that promotes lung cancer cell migration [46]. The capability of targeting a number of genes with diverse functions makes miRNAs ideal candidates to regulate core carcinogenesis process [47–50].

Most onco-miRs or ts-miRs are intragenic miRNAs. Increasing evidences demonstrated links between intragenic miRNAs and host genes in cancer. They act in synergistic or antagonistic ways, which is achieved by miRNA-mediated fine-tuning target gene expression functionally related to host genes [51]. Due to the close functional connection between intragenic miRNAs and host genes, it is necessary and important to interpret the function of these onco-miRs and ts-miRs in the context of host genes. TCGA provides a rich source of multi-dimensional omics data, which gives a unique opportunity to study the expression of intragenic miRNAs and their host genes simultaneously across multiple cancer types. A positively correlated expression patterns would indicate a co-regulation and/or synergistic relationship between host and intragenic miRNAs, while anti-correlated or uncorrelated expression patterns would suggest an independent or antagonistic process.

Known onco-miRs and ts-miRs, specific cancer(s) where they are involved in, their cognate host genes and their expression correlation coefficients in specific cancer(s) are listed in Table 1. Most intragenic onco-miRs and ts-miRs are embedded within intron regions of genes. They exhibited a strong co-expression relationship with their cognate host genes, suggesting co-transcription and/or potential cooperation in regulating cancer development. The results derived from TCGA data sets further validated previous findings. For example, miR-335 harbored within an intron of a protein-coding gene MEST, was known to be co-regulated with MEST by promoter hypermethylation in breast cancer cells [119], HCC [29] and gastric cancer [120]. Consistently, the expression of miR-335 and MEST are highly correlated in TCGA HCC (R = 0.88; FDR = 9.87e-05), breast (R = 0.78; FDR = 0) and gastric cancer (R = 0.79; FDR = 0), further validating of the co-regulation relationship between miR-335 and MEST is needed. MiR-106b-25 cluster, composed of the highly conserved miR-106b, miR-93 and miR-25, is embedded within an intron 13 of MCM7. Petrocca *et al.* [121] reported that the MCM7 and miR-106b-25 cooperate in exerting their oncogenic function through different complementary mechanisms. Consistently, miR-106b-25 and MCM7 are highly co-expressed in TCGA breast cancer (R = 0.68, 0.57, 0.66; FDR = 0, 0, 0), further demonstrating that they are derived from the same primary transcript. Meanwhile, intronic miR-224/452 cluster showed significant
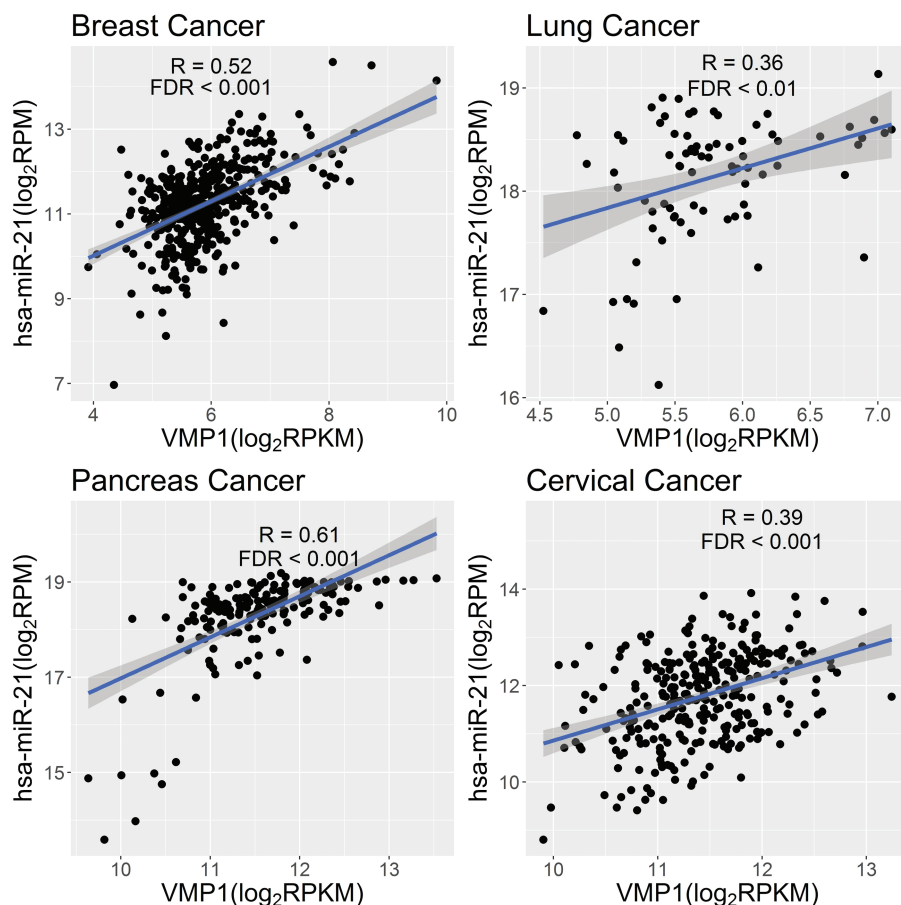
Figure 3. Scatter plot of expression abundance of miR-21 and its host gene VMP1 in multiple cancers.

co-expression with their host gene *GABRE* in prostate cancer (R = 0.58, 0.63; FDR = 0, 0) [122].

Although most intronic onco/ts-miRs are co-expressed with their host genes in cancers, there are some exceptions. For example, expression of miR-30c-1 is not correlated with its host gene *NFYC* (R = 0.06; FDR = 0.23) in prostate cancer, suggesting that it does not originate from the *NFYC* transcription unit. Several cryptic promoters and possible transcription factor binding sites have been detected in the intron regions of *NFYC* where miR-30c-1 is located, indicating miR-30c-1 is transcribed independent from *NFYC* [123]. Consistent with previous findings that miR-1908 is regulated by its own transcription unit rather than co-regulated with its host gene *FADS1* [34], we found that expressions of miR-1908 and *FADS1* are negatively correlated/uncorrelated in glioblastoma and ovarian cancer (R = −0.12, 0.01; FDR = 0.01, 0.87). In addition to known independent transcriptional relationships, TCGA data sets revealed novel uncorrelated/negatively correlated patterns between some intronic miRNAs and their host genes—for example, miR-346 and *GRID1* in liver cancer (R = 0.1; FDR = 0.81), miR-488 and *ASTN1* in gastric cancer (R = −0.2; FDR = 0.006) and miR-489 and *CALCR* in esophageal cancer (R = 0.02; FDR = 0.72). The uncorrelated/negative correlation indicated these miRNAs might have their own transcription mechanism rather than share the promoter as their host genes.

There are three onco/ts-miRs harbored within exonic regions, miR-137, miR-155 and miR-198. MiR-198 is located in the 3′-UTR region of protein-coding gene *FSTL1*, while the other

two miRNAs are encoded in the exon of non-coding genes, *MIR137HG* and *MIR155HG*. MiR-198 is not co-expressed with its host gene *FSTL1* (R = −0.04 in breast cancer; FDR = 0.52), which validates previous finding about their mutually exclusive expression from a single transcript [37]. In contrast, miR-155 is co-transcribed with *MIR155HG* possibly because the gene itself is to generate the miRNA rather than produce other functional unit, leading to the high co-expression pattern in glioblastoma (R = 0.68; FDR = 0).

MiR-21, involved in many cancers, has been reported to be a readthrough miRNA from *VMP1*. The host gene, *VMP1*, was recently found to be involved in the process of tumor metastasis and it plays a vital role in balancing apoptosis and autophagy [38, 124]. miR-21 and *VMP1* are co-expressed across multiple cancer types (R = 0.52, 0.36, 0.61, 0.39; FDR = 0, 0.003, 0, 4.18e-12, in breast, lung, pancreas, and cervical cancers, respectively) (Figure 3). These findings further validated that miR-21 is the byproduct of transcription of *VMP1*.

MiR-34b and BTG4 are co-regulated by a bidirectional promoter [40]. It has been revealed that miR-34b has tumor suppressive ability in breast and prostate cancer [73]. However, TCGA data indicated that there is no correlation between expression of miR-34b and BTG4. Further research is needed to investigate whether this is caused by independent transcription or whether the co-transcription is disrupted by regulation feedback between miRNAs and host genes.

Most interestingly, 15 out of 46 miRNA clusters show cancer-specific co-expression patterns with their host genes, suggesting
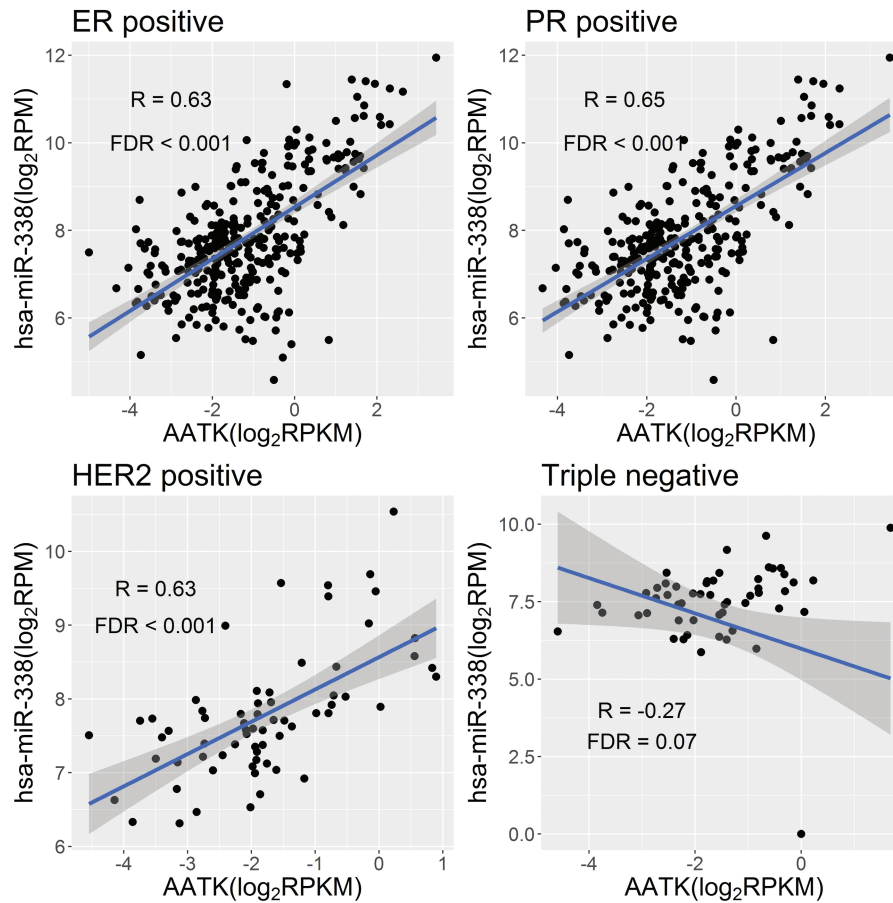
Figure 4. Scatter plot of expression abundance of miR-338 and its host gene AATK in ER/PR/HER2-positive and triple-negative breast cancers.

the common involvement of cancer-specific post-transcription regulation—for example, miR-17-92 cluster, encoded the miR-17, miR-18a, miR-19a/b1, miR-20a and miR-92a1. miR-17-92 cluster, regarded as oncogene, was frequently overexpressed in lymphomas, lung cancer and renal cancer [57–59]. All miRNAs in the cluster showed strong correlation with the host gene C13orf25 in lung cancer (R = 0.49, 0.48, 0.58, 0.55, 0.48, 0.51; FDR = 2.26e-5, 2.89e-5, 1.35e-7, 1.08e-6, 2.82e-5, 9.90e-6), but weak/discordant correlation in renal cell cancer (R = 0.07, 0.01, 0.11, 0.07, 0.16, 0.19; FDR = 0.47, 0.93, 0.22, 0.47, 0.06, 0.02). These results suggest that miRNAs and host genes are co-transcribed from one transcript but renal cancer-specific post-transcription interferes the co-transcriptional regulation. In addition to cancer-specific pattern, some miRNAs even exhibit subtype-specific co-expression with their host genes in one cancer. For example, miR-338 is strongly co-expressed with its host gene AATK in ER/PR/HER2-positive breast cancers (R = 0.63, 0.65, 0.63; FDR = 0, 0, 2.49e-7), but this positive correlation is lost in triple-negative breast cancer (R = −0.27, FDR = 0.07) (Figure 4).

Potential as the therapeutic targets in cancer

As master regulators of cellular processes, miRNAs have become promising therapeutic targets for cancer treatment. Upregulation of the expression of a ts-miR can induce apoptosis and senescence, block cell proliferation, self-renewal, invasion, metastasis, angiogenesis and drug resistance by selectively downregulating multiple genes [125]. Conversely, inhibiting the

function or blocking the expression of an onco-miR in cancer cells can reactivate multiple tumor-suppressor genes, leading to tumor regression or even to tumor eradication [126].

Since most onco-miRs or ts-miRs are intragenic miRNAs, the observation that they are often co-expressed with host genes (Table 1), provides a new strategy to regulate miRNAs expression for cancer therapy. When miRNAs are derived from the transcription of host genes rather than have their own independent promoters, disruption of the expression of host genes or especially epigenetic modification of promoters of host genes is highly likely to change the miRNA expression. For example, miR-335, known to suppress breast cancer cell migration, is highly co-expressed with its host gene in breast cancer (Table 1). Furthermore, miR-335 expression is significantly negatively correlated with the MEST promoter DNA methylation level (R = −0.32; P = 0.0002), suggesting that promoter DNA demethylation would activate miR-335.

Since epigenetic modification is potentially reversible, it has been aggressively pursued as therapeutic strategy. Grady *et al*. [107] showed that there is synchronous epigenetic silencing of the intronic ts-miR miR-342 and its host gene *EVL* in colorectal cancer. Reconstitution of miR-342 activity induces apoptosis in colorectal cancer cells. Daniunaite *et al*. [127] analyzed the promoter methylation level of host genes of miR-155, miR-152, miR-137 and miR-31 in prostate cancer. They found that aberrant promoter methylation of the host genes is prostate cancer-specific. Downregulation of miR-155-5p significantly correlates with the promoter methylation level, which can be used as the

promising diagnostic and/or prognostic biomarker of prostate cancer. The methylation status of host genes of particular miRNA as independent variables or in combinations with other signals might assist physicians in identifying prostate cancer patients with poor prognosis preoperatively. Besides, miR-152 and *COPZ2* were both silenced by hypermethylation in endometrial cancer. They are highly co-expressed in TCGA endometrial cancer data (R = 0.70; FDR = 0; Table 1). Tsuruta *et al.* [67] investigated the relationship between DNA hypermethylation and downregulation of miR-152 and *COPZ2* expression. They found that DNA hypermethylation-mediated silencing of miR-152 and *COPZ2* was a relatively frequent molecular event in endometrial cancer and inhibited cell growth in endometrial cancer cell lines, suggesting the epigenetic silencing of these genes to contribute to endometrial carcinogenesis. The restoration of miR-152 expression in endometrial cancer cell lines was sufficient to inhibit tumor cell growth in vitro and in vivo.

For the specific miRNA with dual identity that either onco-miR or ts-miR, there are different methylation pattern in different cancers. miR-224 and miR-452 are located in the intron 6 of the host gene γ-aminobutyric acid A receptor (*GABRE*). There are significant positive correlations between expressions of miR-224, miR-452 and *GABRE* in previous studies [122] and TCGA data sets across cancer types (R = 0.58∼0.83; FDR = 0; Table 1), demonstrating that these three genes are transcriptionally co-regulated under the same promoter. Cui *et al.* [128] found that miR-224 was significantly upregulated and the CpG island in the promoter region of miR-224 was hypomethylated in lung cancer, suggesting the potent oncogenic role in lung cancer. In contrast, GABRE∼miR-452∼miR-224 locus is downregulated and hypermethylated in prostate cancer, suggesting tumor-suppressive role in prostate cancer [122]. Silencing/activating these miRNAs by epigenetic alterations of cognate host genes can be a potential way for cancer therapy.

Besides DNA methylation, histone acetylation is another way of epigenetic regulation. The ts-miRs of miR-15a/16–1, encoded within an intron region of the non-coding *DLEU2* gene, are downregulated in multiple tumor types while being frequently deleted in CLL. miR-15a/16–1 is believed to be transcribed from the *DLEU2* promoter. Kasar *et al.* [129] revealed that the transcription factor *BSAP* (B-cell-specific activator protein) directly interacts with *DLEU2*, resulting in repression of miR-15a/16–1 expression by negative regulation of the *DLEU2* promoter. Derepression of the *DLEU2* promoter via inhibition of histone deacetylation in combination with *BSAP* knockdown increased miR-15a/16–1 expression, leading to malignant B-cell death. Thus, therapy targeting enhanced host gene *DLEU2* transcription may augment CLL therapy. miR-126, harbored within the intron of the *EGFL7* gene, is downregulated in cancer cell lines and also in primary bladder and prostate tumors. miR-126 and one of the transcripts of *EGFL7* are concomitantly upregulated in cancer cell lines by inhibitors of DNA methylation and histone deacetylation. All these findings demonstrated that epigenetic changes can control the expression of onco/ts-miRs by directly controlling their host genes [130]. The knowledge of common and cancer-specific co-transcription patterns between miRNAs and host genes will help design therapeutic methods, targeting miRNA expression efficiently.

The advantage of miRNAs as therapeutic agents is based on their ability to concurrently target a large number of genes/pathways. However, this ability also causes a major concern on their toxicity due to potential off-target effects. While miRNAs target oncogenic pathways, they modulate other biological processes, which would cause far stronger side effects than gener-

ally appreciated [131]. Additionally, most existing strategies only focus on single miRNA or a family of miRNAs. Since miRNAs act cooperatively in tumor initiation and progression, modulating single miRNA expression has a limited effect. Tumor proliferation can be easily restored through bypassing the miRNA. Strategies to reprogram aberrant miRNA networks in cancer would be more effective [132]. Other challenges for developing miRNA therapeutics include low bioavailability, limited effective delivery and long-term safety [133].

Conclusion

Dysregulation of miRNAs expression plays a critical role in human diseases, especially in cancer. There is a close functional connection and complicated interplay between miRNAs and host genes; therefore, it is essential to study the miRNA regulation in the context of their cognate host genes. Since most onco/ts-miRs are intragenic miRNAs, we studied the co-expression relationship between these miRNAs and host genes based on TCGA data sets. The common and cancer-specific co-expression relationships will facilitate the design of therapeutic methods to silence/activate onco/ts-miRs efficiently by epigenetic alterations of cognate host genes.

Key Points

- Although there is a general understanding of the generation of mature miRNA from primary miRNA, the transcriptional regulation of primary miRNAs is still unclear.
- Recent studies have shown that more than half of miRNAs are located within protein-coding or non-coding genes. Intragenic miRNAs and their host genes either share the promoter or have independent transcription.
- Most ts-miRs and onco-miRs are intragenic miRNAs; therefore, it is necessary and important to interpret their function in the context of host genes.
- The knowledge of common and tumor-specific co-expression patterns between miRNAs and host genes is very helpful for understanding transcriptional and post-transcriptional regulation of miRNAs and further developing miRNA therapeutics in cancer.

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