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Title: miRNA activation is an endogenous gene expression pathway

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Abstract

Transfection of small non-coding RNAs (sncRNAs) molecules has become a routine technique widely used for silencing gene expression by triggering post-transcriptional and transcriptional RNA interference (RNAi) pathways. Moreover, in the past decade, small activating (saRNA) sequences targeting promoter regions were also reported, thereby a RNA-based gene activation (RNAa) mechanism has been proposed. In this regard, Turner and colleagues recently discovered an endogenous microRNA (miRNA) which binds its promoter in order to upregulate its own expression. Interestingly, several miRNA-induced RNA activation (miRNAa) phenomena have since then been identified. My objective here is to introduce the reader into the emergent miRNAa research field, as well as bring together important discoveries about this unexplored transcriptional activation pathway.

Keywords

Double-stranded RNAs; RNA activation; small activating RNAs; microRNA activation; activating miRNAs.

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Main text

In the past decade, it was found that transfection of small double stranded RNA (dsRNA) molecules targeting promoter regions of the E-cadherin, vascular endothelial growth factor (VEGF) and p21 cyclin-dependent kinase inhibitor genes induced target transcriptional activation (Li et. 2006). Shortly after, Janowsky and colleagues (2007) reported short RNA duplexes also able to induce target gene expression of the Progesterone Receptor (PR) gene. Such process was named RNA activation (RNAa), and transfected dsRNAs (19-21 nt) referred to as small activating RNAs (saRNAs) or antigen RNAs (agRNAs) (Li et al. 2006, Janowsky et al. 2007, Schwartz et al. 2008, Yang et al. 2008). The transfection of synthetic saRNAs into different cell lines has allowed to induce selective activation of target developmental genes involved in important cellular phenomena such as cell differentiation and apoptosis (Yang et al. 2008, Zheng et al. 2014), and it has been reported in organisms as diverse as nematodes and mammals (Huang et al. 2010, 2013, Turner et al. 2014). Interestingly, several enzymes required for RNA interference (RNAi) including Dicer and Argonaute (Ago) family members have been also reported to function in RNAa (Li et al. 2006, Huang et

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al. 2013, Place et al. 2008); however, saRNAs exhibit slower and more durable effects when compared to small interfering RNAs (siRNAs) (Li et al. 2006, Janowsky et al. 2007). Functionally, saRNAs function to promote the assembly of the RNA Polymerase II (RNAPII) machinery on target promoters, and subsequently recruit heterogeneous nuclear ribonucleoproteins (hnRNPs), specific coactivators and chromatin remodeling factors which concertedly act to trigger gene activation (Schwartz et al. 2008, Hu et al. 2012, Huang et al. 2012).

In higher eukaryotes, three main types of regulatory small non-coding RNAs (ncRNAs) have been identified: siRNAs, microRNAs (miRNAs) and piwi-interacting RNAs (piwi-RNAs). In particular, miRNAs are known to be evolutionarily conserved ncRNAs which target both DNA and RNA to inhibit gene expression. miRNA genes are first transcribed as long pri-miRNA stem-loop structures which are subsequently cleaved by Drosha and Dicer RNAses III enzymes to generate ~70 nt long precursor miRNAs (pre-miRNAs), and mature miRNAs (21-22 nt), respectively (Lai 2002). In the cytoplasm, miRNAs inhibit translation by mRNA degradation/destabilization and translational repression, while in the nucleus these master regulators control transcript stability and recruit epigenetic remodeling factors in order to induce gene silencing (Catalanotto et al. 2016). Nonetheless, miRNAs can act not only to suppress, but also to induce expression of target genes. In 2008, Place et al. (2008) observed that transfection of synthetic miR-373 mimic targeting the region upstream of the transcription start site of the E-cadherin and the cold-shock domain-containing protein C2 (CSDC2) genes induced transcriptional activation in prostate cancer cell lines. Since then further miRNA-induced activation (miRNAa) phenomena have been reported (Table 1). In murine cell lines, miR-744 and miR-1186 target promoter of the Cyclin B1 (Ccnb1) gene to induce transcriptional activation (Huang et al. 2012). Interestingly, the authors

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reported that knockdown of endogenous miR-744 significantly impaired mRNA expression of Ccnb1, thereby suggesting that miRNAa may represent a naturally occurring mechanism acting under physiological conditions. Likewise miRNAs involved in RNAi, activating miRNAs also require of (1) Drosha and Dicer RNase III enzymes, responsible for miRNA biogenesis, and (2) members of the Argonaute (Ago) family proteins, known to be associated with miRNAs during the formation of the effector RNA-Induced Silencing Complex (RISC) (Huang et al. 2012).

It was not until 2014 that was reported the first experimental evidence of a miRNA acting endogenously to induce target gene expression. In nematodes, Turner and colleagues showed that miRNA lin-4 targets a putative lin-4-complementary element (LCE) situated at its promoter to trigger autoactivation. This evolutionary conserved miRNA (lin-4) was the first miRNA discovered, thereby its effects on gene expression are nowadays well known, especially its ability to regulate the activity of major developmental genes involved in post-embryonic growth (Lee et al. 1993). Importantly, Turner et al. (2014) observed that lin-4 mediates the recruitment of RNAPII to its own promoter and it is sufficient to trigger lin-4 miRNA autoactivation. Relevant research reports on miRNA activation phenomena were made using nematodes as experimental organism models. In *C. elegans*, it also was found that primary transcripts of let-7 miRNA interact with ALG-1 (Argonaute Like Gene 1), and this complex is responsible for processing of mature miRNAs from their corresponding precursors (Zisoulis et al. 2012). Moreover, activating miRNAs have also been reported to induce target host gene expression. In sarcoma cells, the Insulin-like growth factor 2 (IGF2) gene is upregulated by a miRNA localized within one of its introns, miR-483-5p, which targets the 5' UTR of IGF2 mRNA transcripts (Liu et al. 2013). IGF2 is a tissue-specific maternally imprinted gene whose overexpression has been associated

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with tumorigenesis (Ogawa 1993). Thus, miR-483-5p-mediated IGF2 gene expression would indicate that miRNAa plays an important role in the orchestration of complex epigenetic pathways such as involved in the control of gene imprinting. In support to this view, Liu et al. (2013) showed that miR-483-5p induces transcriptional activation through interaction of IGF2 with RHA (also known as DHX9), a RNA helicase which have been recently identified as a key component of the RNA-Induced Transcriptional Activation (RITA) complex (Portnoy et al. 2016).

According to the experimental data, miRNAa does not fit a single model. Matsui et al. (2013) proposed that endogenous miR-589 induces target gene expression of the cyclooxygenase-2 (COX2) and phospholipase A2 (PLA2G4A) genes via a binding loop mechanism which includes gene promoters, nascent promoter COX2 transcript, Argonaute-2 (Ago2) and GW182, the last two being core components of the miRNA-induced silencing complex (miRISC) (Carthew and Sontheimer 2009). It is important to note that the COX2 and PLA2G4A genes are located at a distance of only 149 kb, and they encode enzymes involved in the same inflammatory pathway. Likewise synthetic saRNAs, activating miRNAs may also produce site-specific epigenetic modifications (Li et al. 2006, Janowski et al. 2007, Huang et al. 2012, 2013, Matsui et al. 2013). In HEK293T cell lines, miR-24-1 overexpression is sufficient to trigger histone 3 lysine 27 acetylation (H3K27ac) at target enhancers (Xiao et al. 2017). In contrast, however, miRNAa targeted to TATA-box motifs seem not to involve epigenetic marks at promoters sequences, as reported during activation of the interleukin-2 (IL-2) gene (Zhang et al. 2014b). In this regard, authors propose that miRNAs targeting TATA-box motifs might enhance the processivity of the RNAPII through the stabilization of the transcriptional machinery.

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miRNAs are critical and versatile regulators that function in the nucleus to regulate gene expression, although how they do it is still an open question. In regard to the miRNAa mechanism, it has shown to require: 1- core components of the miRNA silencing pathway (i.e., Ago1, Ago2, Dicer and Drosha enzymes), 2- enrichment of RNAPII at target sites, and 3- epigenetic transcriptional reprogramming (Huang et al. 2013, Place et al. 2008, Huang et al. 2012, Matsui et al. 2013, Dharap et al. 2013, Huang 2017). There are, however, important question about miRNAa, namely, how activating miRNAs act under physiological conditions, in which cellular processes/circumstances they are transcribed, etc. Importantly, advanced insight into RNA-based regulatory mechanisms would allow design effective and switchable strategies for controlling transcriptional networks. For instance, malfunctioning of the RNAi pathway by miRNA deregulation has been found to be associated with progression of tissue-specific tumors and faulty silencing of specific oncogenes, thereby activating miRNAs might be used to produce miRNAa-mediated transcriptional activation of target tumor suppressors. In conclusion, miRNAa is one of the hottest topics in current molecular biology and it is expected that a more deeply understanding about this emergent field render important insights into genome function, as well as promising therapeutic applications.

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Disclosure Statement

The author reports no potential conflict of interest in the publication of this material.

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Table 1. miRNAs reported to target sequences and induce (or maintain) gene expression. The species from which cell lines/lineages have been derived are indicated in parentheses.

miRNA	Target gene sequence/genome region	Cell lines used	Reference
miR-373	E-cadherin (E-cad), cold-shock domain-containing C2 (CSDC2)	PC-3 (human)	Place et al. (2008)
miR-324-3p	RelA	PC12 (rat)	Dharap et al. (2013)
miR-483-5p	Insulin-like growth factor 2 (IGF2)	MHH-ES-1, RD (human); F4328, F4864 (mouse)	Liu et al. (2013)
miR-589	Cyclooxygenase-2 (COX-2)	A549 (human)	Matsui et al. (2013)
miR-370-5p, miR-1180-5p and miR-1236-3p	p21/CDKN1A	T24, EJ, A549 (human)	Wang et al. (2014); Li et al. (2017a)
miR-1236	p21/CDKN1A	786-O, ACHN (human)	Wang et al. (2016)
miR-3619-5p	p21/CDKN1A	DU145, PC3 (human)	Li et al. (2017b)
miR-6734	p21/CDKN1A	HCT-116 (human)	Kang et al. (2016)
let-7i	Interleukin-2 (IL-2)	$CD4^+$ T-cells (human, mouse)	Zhang et al. (2014b)
miR-877-3p	p16/CDKN2A	T24, UM-UC-3 (human)	Li et al. (2016)
miR-4281	FOXP3	$CD4^+$ T-cells (human)	Zhang et al. (2018)
miR-551b-3p	STAT3	SKOV3, IGROV1, IOSE80 (human)	Chaluvally-Raghavan et al. (2016)
miR-24-1	Multiple enhancers	HEK293T (human)	Xiao et al. (2017)
miR-140	long noncoding RNA NEAT1	3T3-L1 (mouse)	Gernapudi et al (2016)
miR-744; miR-1186	Ccnb1/Cyclin B1	NIH/3T3, TRAMP C1 (mouse)	Huang et al. (2012)
miR-H3	Human immunodeficiency virus type 1 (HIV-1)	HEK293T (human)	Zhang et al. (2014a)
lin-4	lin-4 (autoregulation)	Hypodermal seam cells (*C. elegans*)	Turner et al. (2014)

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