Review

Cancer Research

Circular RNA and miR-7 in Cancer

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Abstract

MicroRNAs (miRNA) play important roles in fine-tuning gene expression and are often deregulated in cancer. The identification of competing endogenous RNA and circular RNA (circRNA) as important regulators of miRNA activity underscores the increasing complexity of ncRNA-mediated regulatory networks. Particularly, the recently identified circular RNA, ciRS-7, which acts as a designated miR-7 inhibitor/sponge, has conceptually changed the mechanistic understanding of miRNA networks. As miR-7 modulates the expression of several oncogenes, disclosing the regulation of miR-7 activity will likely advance the understanding of various cancer etiologies. Here, we review the current knowledge about the ciRS-7/miR-7 axis in cancer-related pathways and discuss possible models explaining the relevance of coexpressing miR-7 along with a circRNA inhibitor. *Cancer Res; 73(18); 5609–12. ©2013 AACR.*

Circular RNAs (circRNA) represent a novel class of widespread and diverse endogenous RNAs that regulate gene expression in mammals. We and others recently uncovered the function of one such circRNA, ciRS-7 (also termed CDR1as), as a circular miR-7 inhibitor, which harbors more than 70 conventional miR-7 binding sites (1, 2). Expression of ciRS-7 efficiently tethers miR-7, resulting in reduced miR-7 activity and increased levels of miR-7–targeted transcripts (Fig. 1A), consistent with the microRNA (miRNA) sponge and competing eRNA (ceRNA) hypothesis (3). However, in contrast with classical ceRNAs, ciRS-7 possesses no accessible termini, rendering it resistant to miRNA-mediated RNA destabilization, which is normally facilitated by exonucleolytic decay. This observation has several interesting implications concerning miR-7 biology and toward disclosing the authentic involvement of miR-7 in various disease-related pathways.

ciRS-7 is transcribed in the antisense orientation with respect to the *CDR1* gene, cerebellum degeneration-related antigen 1 (also known as CDR34), which since the late 80s has been implicated as one of two important genes (the other being *CDR2*/*CDR62*) in an autoimmune neurologic disorder (4–6). As researchers have used standard protocols with double-stranded probes to profile *CDR1* mRNA expression in Northern or dot-blotting analyses, these profiles lack strand-specific information and, therefore, most likely represent ciRS-7 expression rather than *CDR1* mRNA expression. Therefore, from data obtained more than 2 decades ago, it is evident that ciRS-7 is expressed primarily in brain, particularly in the cerebellum (4), in agreement with our ciRS-7 profiling analyses (7). Accordingly, expression analyses of various tumor-derived cell lines showed widespread expression of ciRS-7 in neuroblastomas and frequent expression in astrocytoma, renal cell, and lung carcinomas (4), although never to the same extent as normal brain tissue, implying reduced ciRS-7 expression in transformed neuronal cells. However, a clear demarcation in terms of expression was observed between neuroblastoma (N-type) cells with high ciRS-7 expression and substrate adhering (S-type) neuroblastoma variants showing limited expression (6). Collectively, this indicates that adherent properties common for a multitude of cultured cell lines are somehow connected to low ciRS-7 expression and could in part explain the dissonance between tissue and cell lines expression of ciRS-7. Finally, stable overexpression of prion protein PrPC in HEK293 cells was shown to induce ciRS-7 expression and not *CDR1*, as initially thought (8). Hence, PrPC could possibly be involved in the regulation of ciRS-7 and unveiling the function of ciRS-7 in prion disease would be an interesting possibility to pursue.

In the early literature, it was noted that several tandem repeats existed in the *CDR1* locus, especially the recurrence of the hexamer GAAGAC in an 18-nucleotide long unit was emphasized (6). But interestingly, in the antisense ciRS-7, the hexameric complementary motif is part of the miR-7 target sequence. In fact, the approximately 18-nucleotide tandem repeat unit makes up approximately 90% of the entire ciRS-7 sequence leaving very little room for potential alternative functions for this circular molecule. Alternatively, the hexameric repeats could potentially encode a repetitive amino acid sequence within the CDR1 protein. However, although a notable region of hexamers is indeed positioned in-frame, only a subset overlaps with the predicted and annotated open reading frame of *CDR1*, arguing for conserved functionality that is independent of the protein coding potential of *CDR1* mRNA.

Consequently, the ability of ciRS-7 to recruit and interact with miR-7 seems to be the primary, if not the sole, function of

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doi: 10.1158/0008-5472.CAN-13-1568

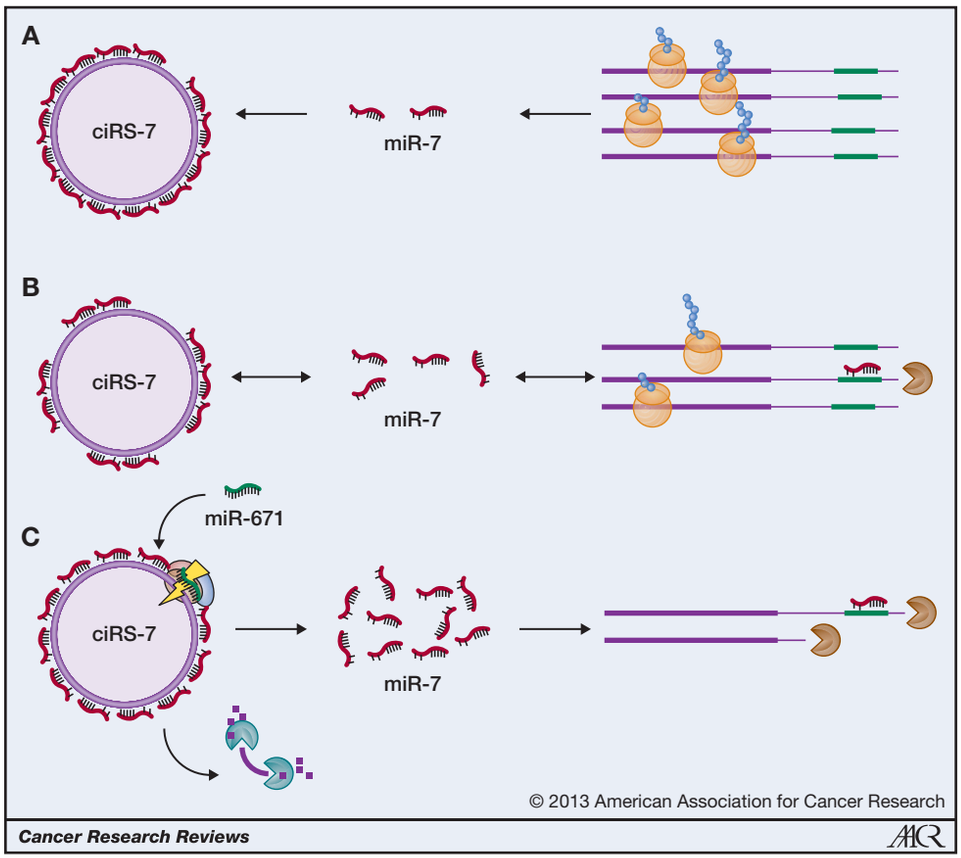

Figure 1. Modes of ciRS-7–mediated regulation of miR-7. A, CiRS-7 functions as a miR-7 inhibitor. Efficient recruitment and inactivation of miR-7 by ciRS-7 results in complete derepression and expression of miR-7–regulated targets. B, ciRS-7 functions as a miR-7 buffer. Competition between miR-7 targets and ciRS-7 allows a balanced target repression and results in buffered miR-7 activity hereby ensuring robustness against transient fluctuations in miR-7 expression. C, ciRS-7 functions as a miR-7 reservoir. In this scenario, release of miR-7 by miR-671–directed cleavage of ciRS-7 results in prompt and efficient repression of miR-7 targets, thus providing immediate and potent spatiotemporal activation of miR-7 activity.

ciRS-7. With that in mind, ciRS-7 must functionally always be contemplated in conjunction with miR-7. In the mouse brain, *in situ* profiling of miR-7 and ciRS-7 showed a remarkable overlap in expression and it is likely that the vast majority of especially brain-expressed miR-7 is tethered to ciRS-7. From an evolutionary perspective, however, miR-7 exists in all animals investigated, whereas ciRS-7 seems to be common for all placental mammals.

To grasp the biologic role of ciRS-7, certainly the regulatory network in which miR-7 participates must be considered. Today, several lines of evidence implicate miR-7 in numerous pathways and diseases: As a direct regulator of α-synuclein, miR-7 has been proposed a role in Parkinson disease (9). Also, inhibition of miR-7 in pancreatic β-cells induced mTOR signaling with subsequent stimulatory effects on cellular proliferation (10). This highlights miR-7 as a putative cause of low β-cell renewal and a possible therapeutic target in diabetes. In addition, miR-7 directly targets and downregulates central oncogenic factors in cancer-associated signaling pathways including EGF receptor (EGFR; ref. 11), IRS-1 (11), IRS-2 (11), Pak1 (12), Raf1 (13), Ack1 (14), and PIK3CD (15), indicating a clear tumor-suppressive role for miR-7. Corroborating such a role for miR-7, it was observed to be the most reduced miRNA in cancer stem-like cells (CSC; ref. 16). CSCs are believed to be an essential player in tumor progression and metastasis (17). Here, the tumor-suppressive role of miR-7 could in part be explained by the concomitant derepression of Kruppel-like factor 4 (16), a progenitor-expressed transcription factor important for stem cell maintenance and prevention of differentiation. In addition, miR-7 indirectly upregulates E-cadherin by targeting insulin-like growth factor 1 receptor (IGF-RI; refs. 18, 19) and focal adhesion kinase (20, 21), resulting in reduced epithelial-to-mesenchymal transition, reduced anchorage-independent growth and suppression of metastasis. Also, in colorectal cancer miR-7 was among the most downregulated miRNAs and it was shown to target the oncogenic YY1 transcription factor ultimately leading to derepression of p53 (22).

Despite overwhelming evidence supporting a tumor-suppressive role of miR-7, the opposite effect has also been reported. For example, lung carcinomas and poor prognosis was found to be associated with miR-7 overexpression (23). In this study, the authors also show that tumor volume in nude mice increased significantly with a concomitant decrease in survival rate when injected with CL1-5 cells expressing miR-7 artificially. Furthermore, inhibition of miR-7 caused reduced proliferation and increased apoptosis in HeLa and lung carcinoma cell lines, respectively (24), suggesting that high miR-7 expression is not necessarily beneficial in terms of inhibiting carcinogenesis. Taken together, these apparently contradictory functions of miR-7 may well be explained by an ambiguous role of miR-7 in regulation of the complex networks of oncogenes and tumor suppressors resulting in a cancer type (and perhaps ciRS-7) dependent outcome.

How is miR-7 expression regulated? The transcriptional regulation of the three miR-7 loci in the human genome is poorly understood. One locus is located within an intron of the ubiquitously expressed hnRNP-K, whereas the other loci are intergenic. Overall, based on miR-7-3p sequencing reads from mirbase, where the three loci are distinguishable, the hnRNP-K–embedded locus seems to be responsible for most miR-7 expression in humans and mouse (here termed mir-7a-1), if assuming that strand-selection ratios between the three loci are similar. It has been indicated, that forced expression of HOXD10 increased miR-7 levels and in turn resulted in a tumor-suppressive phenotype (12), whereas knockdown of Usp18 was shown to elevate miR-7 levels followed by downstream repression of EGFR expression (25). However, upon direct knockdown of EGFR, miR-7-1 topped the list of upregulated miRNAs (23). This effect was surprisingly shown to involve direct association of c-myc with a putative miR-7-1–specific promoter, suggesting an interesting feedback loop with EGFR.

About posttranscriptional regulation, inhibition of miR-7-1 biogenesis by HuR and MSI2 was recently shown (26, 27). Expression of HuR and MSI2 decrease during cortical–neuron differentiation, which, at least in part, explains the concomitant increase in mature miR-7 levels produced from ubiquitous hnRNP-K transcription (28). Also, the SF2/ASF splicing factor was shown to stimulate miR-7 biogenesis, whereas the SF2/ASF mRNA is targeted by miR-7 expression (29), thus establishing an intimate negative feedback mechanism.

Attempts to disclose the biologic role of miR-7 also come from recent analyses of miR-7–knockout fruit flies (30). Although these flies exhibited no apparent phenotype in nonstress conditions, stress revealed a condition where miR-7 had strong positive impact on stress handling by acting in sophisticated feed-forward loops designed to withstand transient fluctuations in expression (as has also been observed for other miRNAs; reviewed in ref. 31). An integral role of miR-7 in cellular robustness and homeostasis resonates well with expression confined to primarily differentiated tissue. Implementing the existence of ciRS-7 in context of cellular robustness, ciRS-7 could possibly serve as a much more sophisticated regulator than merely being a miR-7 inhibitor. In all biologic systems, the competition with mRNA target sites must conform to laws of equilibrium. As a result, high expression of ciRS-7 will in effect exhibit potent inhibition, whereas subtle expression would have intermediate effects. Accordingly, a suboptimal target should be more effectively antagonized by the circular RNA than a strong target site. Hence, one prediction is that the presence of ciRS-7 would specify the target selection of miR-7 toward high-affinity mRNA targets and consequently, ciRS-7 may have a potential to reduce off-target effects of miR-7. Also, highly expressed miR-7–targeted mRNAs would obviously compete with ciRS-7 to a higher degree and thus the inhibitory effect would be less pronounced. Consequently, in terms of equilibrium and robustness, a stable presence of ciRS-7 likely entails buffering of miR-7 activity, i.e., miR-7 activity would be less affected by transient fluctuations in expression and thus upon sudden changes in miR-7 expression, the immediate effects on target gene expression would be automatically rescued by alteration of the ciRS-7–bound/target-bound miR-7 ratio (Fig. 1B). This potentially buffered activity may serve as an additional safety net for miR-7–based networks and, in turn, increase the robustness.

Turnover of ciRS-7 is, at least in part, facilitated by miR-671–dependent Ago2 cleavage (6). The exact fate of bound miR-7 upon degradation of ciRS-7 is currently unknown; however, in line with the "sponge" attribute, often associated with miRNA inhibitors, removal of the ciRS-7 would predict a release and ensuing activation of miR-7. Also, it is currently unresolved whether miRNAs become released and reactivated after sponge association/inhibition; however, based on half-life estimates showing that miRNAs are considerably more long-lived than most mRNAs (32), it is likely that one miRNA is able to target and destabilize several messengers. In addition, evidence from nematodes suggests that miRNAs are even stabilized when encountering their cognate target site (33), which further strengthens the hypothesis of multitargeting capabilities of miRNAs. Therefore, release of miR-7 upon miR-671 cleavage definitely seems a plausible outcome. In such a speculative scenario, miR-671 would act as an activator that liberates miR-7 upon specific and potentially spatiotemporal cues. Thus, the accumulation and storage of miR-7 on ciRS-7 is a putative "miR-7 reservoir" ready to become activated. This would result in a sudden high miR-7 activity ensuring a possible rapid transition between scenarios (Fig. 1C). Alternatively, miR-671 primarily avoids accumulation of ciRS-7, which, due to its circular structure, otherwise would exhibit a very long half-life, or to simply remove ciRS-7 completely in tissues where ciRS-7 is unwanted. However, as miRNA-mediated cleavage is an enzymatic process (34), it is likely that even low miR-671 levels would be sufficient for notable knockdown of ciRS-7. Another possibility is that miR-671 is confined primarily to particular subcellular locales. In this context, ciRS-7 uses the role as a miR-7–trafficking vehicle and miR-7 activity would be spatially controlled to certain intracellular compartments. Transient expression of pri-miR-671 results in predominantly nuclear localization of the mature miR-671 (7) and therefore, nuclear clearance of ciRS-7 could be a subcellular effect of miR-671. In any event, miR-671 and ciRS-7 seem to be coupled evolutionarily, i.e., both restricted to placental mammals.

Finally, considering the high miR-7 and ciRS-7 expression in brain, a considerable amount of RNA-induced silencing complexes (RISC) would be tethered by miR-7 to the circular RNA. Thus, the abundant miR-7/ciRS-7 association may significantly affect the cellular pool of available RISC components. Consequently, miRNA activity and regulation by miRNAs in general would be less pronounced in miR-7/ciRS-7–expressing tissues.

Taken together, in placental mammals, miR-7 is closely coupled to ciRS-7 and fine-tuning of the miR-7/miR-671/ciRS-7 axis will likely play profound roles in diseases such as cancer. Hence, the notion that miRNAs can exist either as active (free/target-bound) or inactive (sponge-bound), complicates the interpretation of bulk miRNA profiling experiments in studies correlating miRNA expression levels to various diseases. The emerging roles of the highly complex

network of ceRNAs, which communicate via miRNA and the overwhelming number of identified circular RNA structures in humans and other organisms prompts for exciting new avenues of research to uncover the full biologic functions of these ncRNAs in cellular regulation and disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Writing, review, and/or revision of the manuscript: T.B. Hansen, J. Kjems, C.K. Damgaard

Grant Support

This work was supported by the Lundbeck Foundation and the Danish Council for Independent Research.

Received May 31, 2013; revised July 1, 2013; accepted July 1, 2013; published OnlineFirst September 6, 2013.

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