MINI REVIEW

ceRNA Cross-Talk in Cancer: When ce-bling Rivalries Go Awry

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ABSTRACT The cancer transcriptome is characterized by aberrant expression of both protein-coding and noncoding transcripts. Similar to mRNAs, a significant portion of the noncoding transcriptome, including long noncoding RNAs and pseudogenes, harbors microRNA (miRNA)-response elements (MRE). The recent discovery of competitive endogenous RNAs (ceRNA), natural decoys that compete for a common pool of miRNAs, provides a framework to systematically functionalize MRE-harboring noncoding RNAs and integrate them with the protein-coding RNA dimension in complex ceRNA networks. Functional interactions in ceRNA networks aid in coordinating a number of biologic processes and, when perturbed, contribute to disease pathogenesis. In this review, we discuss recent discoveries that implicate natural miRNA decoys in the development of cancer.

Significance: Cross-talk between ceRNAs through shared miRNAs represents a novel layer of gene regulation that plays important roles in the physiology and development of diseases such as cancer. As cross-talk can be predicted on the basis of the overlap of miRNA-binding sites, we are now one step closer to a complete functionalization of the human transcriptome, especially the noncoding space. *Cancer Discov; 3(10); 1113–21. ©2013 AACR.*

INTRODUCTION

Diseases such as cancer are often associated with aberrant transcriptomes. This is not limited to the production of abnormal levels of protein-coding mRNAs, but also includes deregulated expression of the noncoding dimension of the human genome. Intriguingly, recent analyses of the human transcriptome have revealed that a mere 2% of the genome encodes protein-coding transcripts, even though over three quarters of the genome is transcribed (1, 2). The function of several classes of noncoding transcripts, such as tRNAs, small nucleolar RNAs (snoRNA), and microRNAs (miRNA, miR), is relatively well understood. However, the functions of a huge portion of the genomic "dark matter" remain to be unraveled. This undertaking is, at least in part, hindered by the lack of systematic means to functionally annotate the noncoding transcriptome. As the noncoding space likely plays critical roles in promoting cancer, a better understanding of its functions in physiology and disease is imperative for the development of novel therapeutic strategies.

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The ceRNA Hypothesis

miRNAs—gene-regulatory noncoding RNAs that are expressed in plants, fungi, animals, and viruses (3)—direct the RNA-induced silencing complex (RISC) to miRNA-response elements (MRE), thereby repressing protein production by either inhibiting translation or destabilizing the mRNA (4, 5). MREs are localized on the 3′ untranslated region (UTR), coding sequence (CDS), and the 5′ UTR, and, in addition to being found on mRNAs, can also be found on non–protein coding transcripts such as pseudogenes and long noncoding RNAs (lncRNA; ref. 6). Importantly, each miRNA has numerous RNA targets, and the vast majority of RNA molecules harbor several MREs and are thus repressed by different miRNAs. This target multiplicity has led to the hypothesis that different RNAs (either pseudo targets or legitimate targets) compete for limited pools of miRNAs (7, 8), thus acting as competitive endogenous RNAs (ceRNA; Fig. 1A). The ceRNA hypothesis postulates that any RNA transcript that harbors MREs can sequester miRNAs from other targets sharing the same MREs, thereby regulating their expression (Fig. 1B and C). Importantly, the ability of two transcripts to cross-regulate each other can be bioinformatically predicted on the basis of the MRE overlap, that is, the MREs that they have in common. The ceRNA hypothesis thus proposes two very intriguing possibilities. First, a major function of noncoding RNAs such as lncRNAs and pseudogenes that harbor MREs may be to act as endogenous miRNA decoys. As over two thirds of the human genome is transcribed (1), the ability to predict putative ceRNA interactions may allow functionalization of a

KEY CONCEPTS

- Competitive endogenous RNAs (ceRNA) share miRNA-response elements (MRE) and sequester common microRNAs (miRNA), thereby regulating each other's expression.
- mRNAs, pseudogenes, long noncoding RNAs (lncRNA), and circular RNAs (circRNA) may all serve as ceRNAs.
- ceRNAs and miRNAs form complex regulatory networks termed ceRNETs.
- Perturbations of critical nodes in ceRNETs may disrupt the balance of the network and thereby contribute to disease development.

sizeable portion of the human transcriptome. Second, besides serving as a blueprint for protein production, mRNAs may be involved in the posttranscriptional regulation of other transcripts.

ceRNA Activity Is Evident in Different Biologic Contexts

Artificial sponges have been used for several years to investigate the function of miRNAs. Such sponges harbor one or more complementary binding sites to a miRNA family and are delivered to cells or transgenic animals, where they sequester the miRNAs of interest and thereby abolish their function (9). Experimental evidence for natural sponges (ceRNAs) was found in several species and contexts and includes a variety of RNA molecules, such as mRNAs, pseudogenes, and long intergenic noncoding RNAs (lncRNA). The *Arabidopsis thaliana* noncoding RNA *IPS1* sequesters miR-399 from its target mRNA *PHO2* and thereby regulates shoot phosphate content (10). This process, termed "target mimicry," relies on imperfect complementarity of miR-399 and *IPS1* at the miRNA cleavage site, which results in sequestration of the miRNA (10). In zebrafish, miR-430 targets both the Nodal agonist *squint* and the Nodal antagonist *lefty* (11), and, thus, both agonist and antagonist can be considered to be natural sponges for each other that ensure balanced Nodal signaling. Similarly, the ligand *Sdf1a* and its sequestration receptor *Cxcr7b* compete for miR-430 to control germ cell migration in the zebrafish gonad (12). The first evidence for ceRNA activity in mammalian cells came from the analysis of the *PTEN* pseudogene in regulating its parental gene (13) and is discussed in detail below. In addition, Cesana and colleagues (14) ascribed a ceRNA function to a lncRNA, *linc-MD1*, which liberates the differentiation factors *MEF2C* and *MAML1* from repression by decoying miR-135 and -133, respectively, thus controlling muscle differentiation. Duchenne muscle cells exhibit reduced levels of *linc-MD1*, suggesting that this axis plays a role in the pathogenesis of Duchenne muscular dystrophy (14). Another lncRNA, *linc-RoR*, controls self-renewal of human embryonic stem cells by sequestering miR-145 from the stemness factors *OCT4*, *NANOG*, and *SOX2* (15). On the basis of these experimental findings, it was hypothesized that a myriad of natural sponges may exist and play critical roles in biologic processes (16). Thus, competition for shared miRNAs

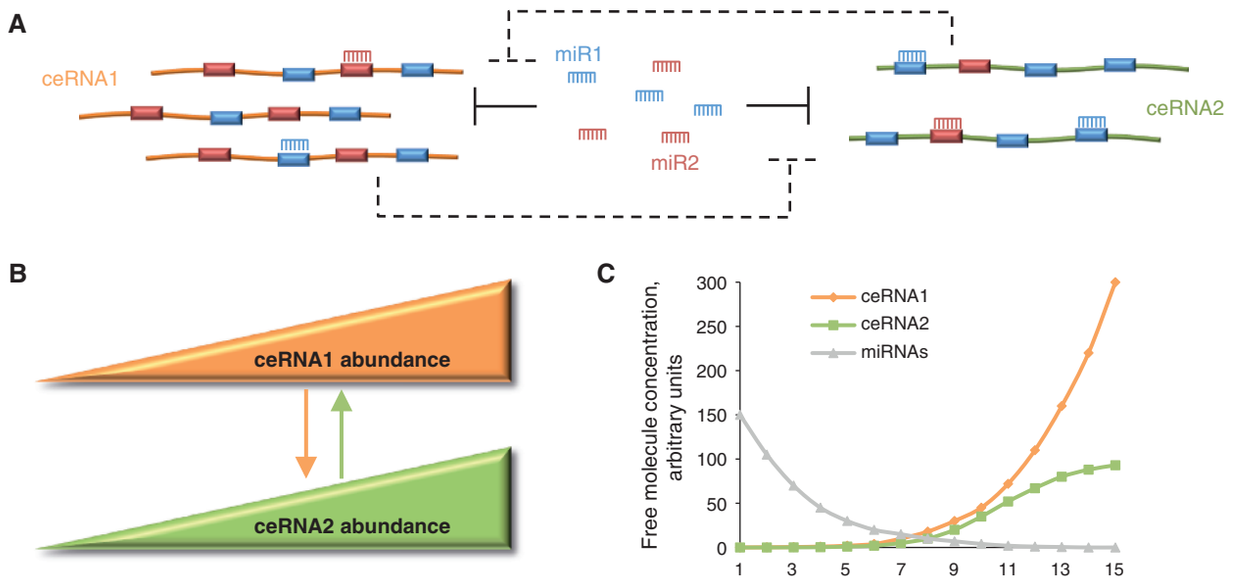

Figure 1. The ceRNA concept. **A,** the schema outlines the concept of ceRNAs. Two transcripts, ceRNA1 in orange and ceRNA2 in green, have two different MREs (blue and red boxes, complementary to the miRNAs miR1 and miR2, respectively) in common. Thus, miR1 and miR2 repress both ceRNAs. When miR1 and miR2 target ceRNA1 and are therefore eliminated from the pool of available miRNA, ceRNA1 effectively inhibits the repressive activity of miR1 and miR2 toward ceRNA2 (dashed inhibition arrow). As ceRNA cross-talk is, in theory, reciprocal, binding of miR1 and miR2 to ceRNA2 inhibits their activity toward ceRNA1. **B,** changing the abundance of one ceRNA will lead to a similar effect in the levels of the other ceRNA; that is, upregulation of ceRNA1 will lead to increased levels of ceRNA2. **C,** deregulation of one ceRNA results in changes in the pool of available, unbound ceRNAs and miRNAs (adapted from ref. 33). Increased expression of ceRNA1 will first lead to a decrease in unbound miRNA molecules. Once the majority of miRNA molecules are bound, the levels of unbound, unrepressed ceRNA1 and ceRNA2 can increase.

may lend robustness to signaling pathways during development and physiology, and it is likely that a multitude of such functional interactions remains to be discovered.

Interestingly, ceRNAs are also found in lower organisms. Viruses have evolved to express ceRNAs to ablate antiviral host miRNAs, as discovered by Cazalla and colleagues (17), who identified miR-27–binding sites in the Herpesvirus saimiri noncoding RNA *HSUR1*. Indeed, virally transformed T cells expressed lower levels of miR-27 and increased levels of the miR-27 target *FOXO1* (17). Moreover, the virally expressed and highly abundant *m169* transcript binds to and promotes the degradation of host miR-27, a process that is important for the replication of murine cytomegalovirus (18, 19). The examples described above indicate that endogenous miRNA decoys have important functions in various biologic processes and cell types, and it is likely that ceRNAs can be found in all organisms that use miRNAs to regulate gene expression. Given the prominent roles of ceRNAs in physiology, it is conceivable that their deregulation is a common occurrence in, and promotes progression of, various diseases such as cancer.

Pseudogenes as ceRNAs in Cancer

Pseudogenes were widely considered "junk" DNA and genomic relicts of evolution because the vast majority of pseudogenes do not encode for functional proteins. Pseudogenes can develop through several mechanisms: gene duplication gives rise to unprocessed pseudogenes that contain introns, 5′ UTRs, and 3′ UTRs, and are under the control of similar promoter elements as their parental genes. Processed pseudogenes form through retrotranscription of spliced mRNA into DNA, which is then reintegrated into the genome. Processed pseudogenes may contain 5′ and 3′ UTRs, but their expression is regulated by promoter and enhancer elements that differ from their parental genes. Finally, unitary pseudogenes develop *de novo* and have no protein-coding counterparts. The three classes of pseudogenes harbor mutations that disrupt the start codon and prevent the production of a functional protein (20). Processed and unprocessed pseudogenes typically maintain a high sequence homology with their parental genes, and thus also display a high degree of MRE overlap (i.e., the number and identity of MREs) with their parental genes. Given this notable MRE overlap, pseudogenes may, at least theoretically, constitute perfect endogenous miRNA sponges.

This theory was first interrogated by examining the PTEN pseudogene, *PTENP1*. PTEN is a haploinsufficient tumor suppressor that is commonly lost or mutated in a variety of human malignancies, such as cancers of the prostate, breast, and colon, glioblastoma, and melanoma (21). Importantly, even a 20% reduction in PTEN levels can elicit a cancer phenotype in mice (22, 23). The proximal 3′ UTR region of *PTENP1* is highly homologous to the proximal *PTEN* 3′ UTR and contains numerous seed matches for miRNAs that were previously validated to repress *PTEN* expression (13), suggesting that *PTENP1* could act as a ceRNA for *PTEN*. Indeed, *PTENP1* is repressed by several validated *PTEN*-targeting miRNAs, and overexpression and RNA interference silencing experiments confirmed that *PTENP1* posttranscriptionally regulates expression of PTEN (13). Notably, *PTENP1* expression levels positively correlate with those of *PTEN* in human tissues and prostate cancer, and genomic losses of the locus containing *PTENP1* occur in human colon cancer and are accompanied by reduced *PTEN* expression (13). Genomic losses of *PTENP1* were also reported in melanoma (24), a cancer known to be promoted by reduction of PTEN expression (25), further emphasizing the tumor-suppressive function of *PTENP1*. Thus, the ceRNA activity of *PTENP1* contributes to posttranscriptional regulation of *PTEN*, and alterations of *PTENP1* expression levels or miRNA decoy activity may lead to modest variation in *PTEN* levels to promote cancer development.

A recent study added a further layer of complexity to the regulation of PTEN by *PTENP1*. Johnsson and colleagues (26) reported that the *PTENP1* locus encodes two antisense transcripts, *PTENpg1* antisense RNA (asRNA) α and β, that differ in their transcriptional start sites but overlap with the *PTENP1* transcript. Intriguingly, these two *PTENpg1* asRNA transcripts have opposing functions. *PTENpg1* asRNA α recruits the chromatin-remodeling factors EZH2 and DNMT3a to the *PTEN* promoter, where they catalyze the addition of a repressive H3K27me3 chromatin mark (26). The α asRNA isoform thus negatively regulates *PTEN* expression independent of the *PTENP1* sense transcript. Conversely, *PTENpg1* asRNA β associates with *PTENP1* sense to increase the stability of the *PTEN* pseudogene transcript. The 3′ UTR of *PTENP1* lacks a robust polyA tail, and this RNA element is likely provided by *PTENpg1* asRNA β in the RNA–RNA complex to increase RNA stability (26). Altering the levels of *PTENpg1* asRNA β changed the expression of *PTENP1* as well as *PTEN*, indicating that *PTENpg1* asRNA β promotes the miRNA decoy function of *PTENP1* (26). The fact that the *PTENP1* locus is lost in cancer (13, 24) may suggest that the stimulatory ceRNA function of the *PTENP1*–*PTENpg1* asRNA β RNA complex is more critically involved in regulating *PTEN* expression than the inhibitory function of *PTENpg1* asRNA α. These findings suggest that the *PTEN* pseudogene is not just a mere artifact of evolution, but rather a regulated modulator of PTEN expression that, when perturbed, may contribute to cancer formation.

It is currently unknown whether *PTENP1* is an exception among pseudogenes, and the question of whether other pseudogenes have similar miRNA-dependent regulatory functions toward their parental genes and possibly other genes with which they share miRNAs remains to be investigated. Notably, however, overexpression of the 3′ UTR of the *KRAS* pseudogene *KRAS1P* increases KRAS levels and promotes cells growth (13). Moreover, the *KRAS* and *KRASP1* transcripts show a positive correlation of expression, and the *KRASP1* locus is amplified in human cancers (13). In addition, several other pseudogenes, such as the *OCT4*, *FOXO*, *NPM*, and *CDK4* pseudogenes, were shown to share binding sites for common miRNAs with their parental genes (13), suggesting that gene regulation by miRNA-sequestrating pseudogenes could be a frequently occurring phenomenon. Interestingly, RNA-seq analyses revealed that a myriad of pseudogenes is expressed in human cells and cancers. Some of these pseudogenes are expressed almost ubiquitously, whereas others are found only in a subset of the analyzed cell types or are specific to a single cell or cancer type (27). Although many of these pseudogenes

may have no function or miRNA-independent functions, a large fraction of these likely represents ceRNAs. Further work is required to identify pseudogenes with the ability to decoy miRNAs from their parental genes, to determine whether the abundance of such pseudogenes relative to their parental genes permits ceRNA cross-talk, and to analyze whether perturbed expression of these pseudogenes contributes to cancer development using *in vitro* and, where appropriate, mouse modeling approaches.

mRNAs as ceRNAs in Cancer

Similar to pseudogenes, mRNAs may promote cancer by regulating the expression of tumor suppressors and oncogenes in a miRNA-dependent manner, provided that they share MREs that permit ceRNA cross-talk. Intriguingly, miRNA sequestration is independent of the protein-coding function of mRNAs, thus allowing genes to participate in diverse and potentially even opposing biologic processes through the mRNA transcript and the protein that they encode. Our laboratory used a computational approach to identify mRNAs that contain at least seven of 10 MREs that are complementary to validated *PTEN*-targeting miRNAs in their 3′ UTRs, an approach termed mutually targeted MRE enrichment (28). This analysis identified numerous putative *PTEN* ceRNAs, two of which, *CNOT6L* and *VAPA*, were validated to regulate PTEN expression in a miRNA-dependent and protein coding–independent manner (28). Moreover, silencing *CNOT6L* and *VAPA* induced AKT signaling and promoted cell proliferation (28). Using a different bioinformatic approach, Sumazin and colleagues (29) identified multiple mRNAs with putative ceRNA activity toward *PTEN*, and experimental validation confirmed their 3′ UTR–dependent regulation of *PTEN*. Interestingly, both studies found reduced expression levels of *PTEN* ceRNAs in human cancers (28, 29). In addition, a forward genetic screen in a $BRAF^{V600E}$–driven mouse model of melanoma revealed that a remarkable 10% of the identified hits in protein-coding genes were putative ceRNAs for *PTEN* (30). *CNOT6L* was also identified in this melanoma screen, and silencing of *AFF1*, *JARID2*, *MBNL1*, *RBM9*, *TNRC6a*, *TNRC6b*, and *ZEB2* decreased PTEN levels (30), suggesting that these transcripts could function as *PTEN* ceRNAs and, thus, tumor suppressors *in vivo*. One putative *PTEN* ceRNA, the *ZEB2* mRNA, was validated to regulate PTEN levels through a miRNA-dependent and protein coding–independent mechanism (30). As the ZEB2 protein possesses oncogenic properties by promoting epithelial–mesenchymal transition (31), this represents an intriguing example of a gene encoding a mRNA and a protein with unrelated functions. The potency of concurrent genetic lesions in BRAF and PTEN has been shown in mice, and even heterozygous loss of PTEN significantly accelerated $BRAF^{V600E}$–induced melanoma development (32). Therefore, moderate changes in PTEN levels elicited by loss of *PTEN* ceRNA expression are likely to contribute to melanomagenesis in the context of a $BRAF^{V600E}$–initiating mutation. Whether aberrant ceRNA-mediated regulation of PTEN promotes cancer in other cell types or cancer-sensitizing genetic backgrounds remains to be determined in mouse models. Moreover, whether haplosufficient tumor suppressors are similarly sensitive to aberrant ceRNA regulation will be an interesting topic for future research. Computational analyses suggest that aberrant expression of numerous mRNAs with putative ceRNA activity correlates with neoplastic phenotypes (29, 33). However, both their ability to decoy miRNAs and their potential to promote cancer remain to be experimentally validated.

3′ UTRs as ceRNAs in Cancer

Another important implication of the ceRNA theory pertains to the role of 3′ UTRs in cancer and other diseases. The 3′ UTRs of protein-coding transcripts are critical for mRNA stability and posttranscriptional regulation, and typically contain MREs for several different miRNAs. In light of the ceRNA hypothesis, 3′ UTRs may not only regulate the stability of their own transcripts in *cis*, but also likely decoy miRNAs from transcripts with shared MREs, thereby regulating such transcripts in *trans*. This, in turn, suggests that mutations or changes in abundance, structure, or length of 3′ UTRs may affect their ability to sponge miRNAs. Curiously, alternative polyadenylation has been observed, leading to 3′ UTR lengthening during embryogenesis (34), and 3′ UTR shortening in proliferating cells (35) and in cancer (36). Changes in 3′ UTR length alter the interaction of miRNAs with such transcripts and affect protein output (35, 36). In addition, due to a reduced number of MREs, 3′ UTR shortening will also modify the ability of these mRNAs to compete for miRNAs and function as ceRNAs. Hence, by alternative polyadenylation, oncogenes may circumvent miRNA-mediated reduction in their abundance and, as a side effect, no longer sequester tumor suppressor–targeting miRNAs. The analysis of such alternative poly-adenylation holds promise to reveal additional facets of ceRNA activity and their contribution to cancer biology.

Intriguingly, Mercer and colleagues (37) recently reported that numerous 3′ UTRs may be expressed independently of their normally associated CDSs, a process that is developmentally regulated in a cell type- and stage-specific fashion. Only a minority of independently expressed 3′ UTRs seem to be generated by *de novo* transcription, and a mechanism involving posttranscriptional cleavage followed by CDS degradation is more likely (ref. 37; Fig. 2A). Future work will reveal how such 3′ UTR transcripts are produced. Nevertheless, their occurrence indicates that 3′ UTRs possess genetic information in addition to regulating stability and translation of their normally associated CDSs. Independently expressed 3′ UTRs may have two transacting ceRNA functions (Fig. 2B). First, they could modulate the expression of the CDS with which they are normally associated. As the sequence and, hence, the number and identity of MREs is identical between the CDS-associated and independently expressed 3′ UTR isoforms, such ceRNA regulation could be extremely potent. Second, independently expressed 3′ UTRs may act as miRNA sponges for and affect expression of numerous other transcripts with which they compete for common miRNAs.

Several reports have described that expression of 3′ UTRs without their associated protein-coding sequences modulates differentiation and proliferation of mammalian cells (38–42). However, as these studies were carried out before the advent of the ceRNA hypothesis, it is unclear whether these

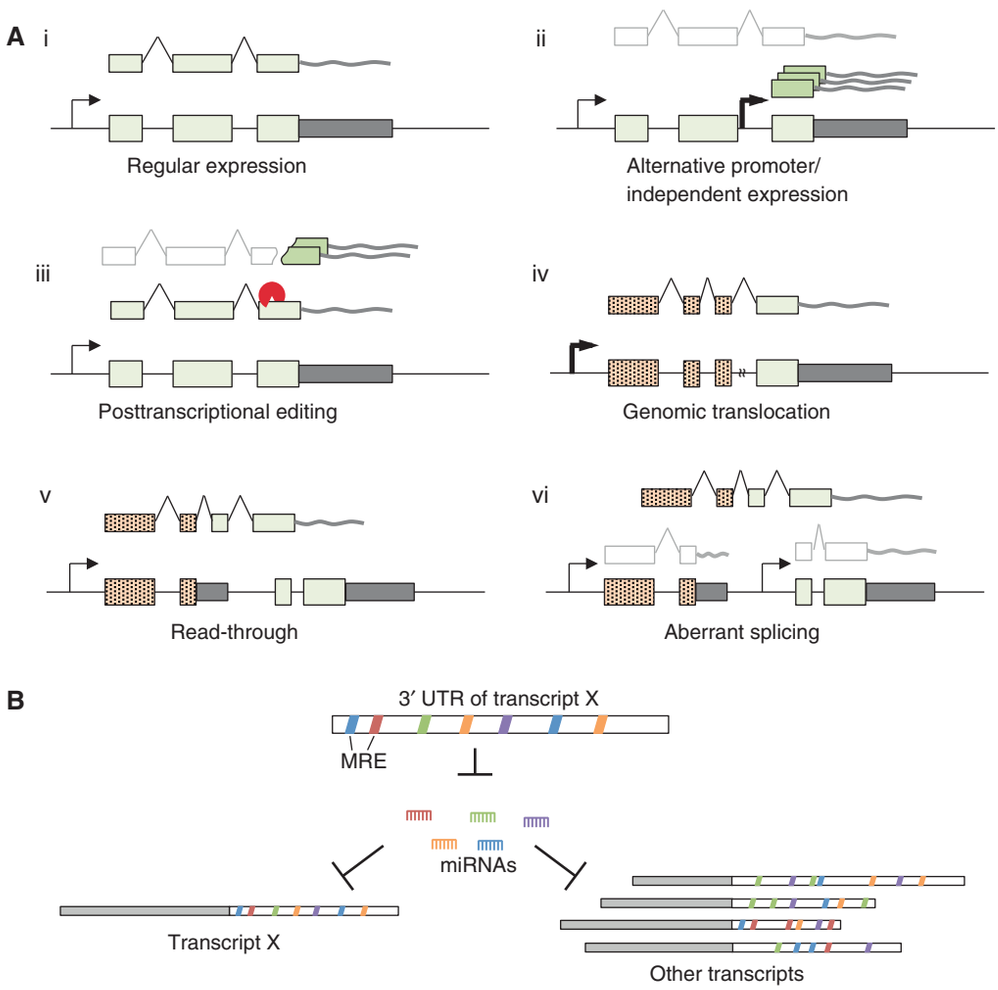

Figure 2. 3′ UTRs as ceRNAs. **A,** independently expressed 3′ UTRs can arise through various mechanisms. **i,** 3′ UTRs are typically associated with the CDSs of their genes, but they nevertheless could serve as ceRNAs for other transcripts. **ii,** alternative promoters may drive independent expression of 3′ UTRs. **iii,** posttranscriptional cleavage of transcripts may dissociate the CDS from its 3′ UTR. **iv,** genomic translocations resulting in fusion genes may place 3′ UTRs under the control of foreign promoters. **v,** transcriptional read-through of two adjacent genes could result in chimeric transcripts and abnormal levels of the downstream transcript. **vi,** aberrant trans-splicing could join two unlinked transcripts, also resulting in chimeric transcripts. **B,** independently expressed 3′ UTRs can either serve as miRNA sponges to derepress expression of their CDS-associated counterparts, or other transcripts with which they share MREs.

3′ UTRs act as miRNA decoys to regulate the expression of their CDS-containing counterparts or other transcripts. More recently, Lee and colleagues generated transgenic mice overexpressing the 3′ UTR of *versican*. In these mice, the *versican* 3′ UTR potentially sequesters miR-199a* to increase protein expression of versican and another miR-199a* target, fibronectin, resulting in abnormal adhesion of the liver to its surrounding organs or the inner body wall (43). In an additional study, the *versican* 3′ UTR was shown to decoy four miRNAs from *versican* and three each from *fibronectin* and *CD34*, and *versican* 3′ UTR transgenic mice developed hepatocellular carcinoma (44). Moreover, ectopic expression of the *versican* 3′ UTR in HepG2 cells enhances transformation *in vitro* and tumor growth in xenografts *in vivo* (44). Aberrant expression of versican, fibronectin, and CD34 is associated with cancer; however, other genes are likely affected by *versican* 3′ UTR overexpression, and these studies do not provide functional evidence that the *versican* 3′ UTR promotes hepatocellular carcinoma by deregulating expression of these three genes. Indeed, overexpression of the *versican* 3′ UTR in the mouse breast carcinoma cell line 4T1 enhances expression of the tumor suppressors PTEN and RB1, leading to reduced proliferation and tumor growth (45). Thus, the effect of deregulated expression of the *versican* 3′ UTR seems to be context-dependent and is likely modulated by the abundance of mRNAs with shared MREs as well as by the pool of miRNAs for which they are in competition.

Other examples of 3′ UTRs functioning as miRNA decoys include the *nephronectin* 3′ UTR, which affects the abundance of β-catenin and GSK3β to promote differentiation of the osteoblast progenitor cell line MC3T3-E1 (46). In addition, ectopic expression of the *CD44* 3′ UTR in the human breast cancer cell line MT-1 relieves repression of CD44

and CDC42 by sequestering at least three shared miRNAs (47). This resulted in reduced proliferation but elevated tube formation *in vitro*, and the formation of smaller, more vascularized tumors in xenograft experiments (47). In a different human breast cancer cell line, MDA-MB-231, overexpression of the *CD44* 3′ UTR enhances cell motility and invasion and promotes metastatic spread *in vivo* (48). *Collagen type 1α1* and *fibronectin* were also among the mRNAs affected by the *CD44* 3′ UTR, suggesting that, similar to the *versican* 3′ UTR, the *CD44* 3′ UTR regulates expression of several protein-coding transcripts (48). Which of these transcripts promote the observed phenotypes in what context awaits further investigation.

Finally, the *PTEN* 3′ UTR, which is among those expressed independently of their CDS (37), affects expression of *PTEN* (13); however, the incidence and abundance of independently expressed *PTEN* 3′ UTR remains to be determined. Taken together, these studies provided evidence that 3′ UTRs have the ability to act as ceRNAs in certain contexts and that their deregulation can facilitate cancer development and progression.

Aberrant 3′ UTR Expression by Genomic Translocation, Alternative Splicing, and Read-Through

An alternative means to express 3′ UTRs independently of their CDS is by genomic translocations that fuse two unrelated genes. This may place a low abundance 3′ UTR under the control of a strong promoter or vice versa (Fig. 2A). Genomic translocations are common in hematologic malignancies and also occur in solid tumors, thus warranting investigations into the capability of their transcripts to serve as cancer-promoting ceRNAs. Other processes leading to aberrant expression of 3′ UTRs include alternative trans-splicing as well as read-through transcription of conjoined genes. Such events result in chimeric transcripts that may be generated independently of their parent genes' transcription. Notably, expression of hundreds of chimeric transcripts has been detected in prostate cancer samples, and their abundance is increased compared with benign control tissue (49). A key role of chimeric transcripts may be to serve as miRNA decoys, and future work will assess their functionality and potency as ceRNAs.

lncRNAs and circRNAs as ceRNAs with Potential Roles in Tumorigenesis

3′ UTRs, CDSs, and 5′ UTRs of mRNAs are peppered with MREs and, hence, many, if not most, mRNAs have the potential to engage in ceRNA cross-talk. Intriguingly, other RNA species such as lncRNAs have recently begun to emerge as natural miRNA decoys. Indeed, lncRNAs are extensively targeted by miRNAs (3, 6), suggesting that they may serve as ceRNAs. The lncRNA highly upregulated in liver cancer (*HULC*) promotes liver cancer (50) and, interestingly, regulates its own expression in a ceRNA-dependent feedforward loop (51). *HULC* expression is induced by protein kinase A (PKA)–mediated phosphorylation of CREB followed by phospho-CREB recruitment to the *HULC* promoter. The *HULC* transcript decoys miR-372 from *PRKACB*, the catalytic subunit of PKA, thus completing this regulatory circuit (51).

A novel class of RNA molecules, termed circular RNAs (circRNA), were shown by two recent studies to act as endogenous ceRNAs. circRNAs form through head-to-tail splicing of exons within the same transcript and thus include intronic and exonic sequences. Such transcripts are very stable, and there may be thousands of circRNAs encoded in the human genome that are expressed in context-dependent manners and are found in normal and malignant cells (52–54). Memczak and colleagues (52) and Hansen and colleagues (55) showed that the circRNA encoded by the cerebellar degeneration–related protein 1 (*CDR1*) gene, which they termed *CD1Ras* and *ciRS-7*, respectively, contains dozens of miR-7 seed matches and is loaded with miRNA effector complexes. The circRNA is insensitive to miR-7–mediated destabilization and serves as an endogenous miRNA sponge that regulates expression levels of miR-7 target genes (52, 55). Moreover, the testis-specific circRNA sex-determining region Y circRNA acts as a ceRNA by decoying miR-138 (55). Given the sheer abundance of circRNAs, their stability, and the potential number of MREs that they may contain, circRNAs are bound to become a potent class of ceRNAs with important roles in both normal physiology and diseases.

ceRNETs

Most studies that reported ceRNA cross-talk between RNA molecules focused on binary and sometimes reciprocal interactions that occur between two transcripts and are mediated by one or more miRNAs. Most transcripts, however, contain numerous MREs for different miRNAs. In addition, miRNAs typically repress multiple target transcripts. This complexity suggests that miRNAs and their targets are connected in complex ceRNA networks, or ceRNETs. ceRNETs likely contain smaller subnetworks where the nodes (i.e., the ceRNAs) are linked by a higher number of connections (i.e., miRNAs), and ceRNA cross-talk may be more efficient in such subnetworks (Fig. 3A). Using a computational approach, Sumazin and colleagues (29) described an extensive ceRNA network in glioblastoma containing approximately 7,000 putative ceRNAs and near a quarter of a million miRNA-mediated interactions. This ceRNET consists of many highly interconnected subnetworks that show a strong positive correlation of expression of their individual ceRNA components and contain numerous transcripts of cancer-associated genes (29). In addition, our group bioinformatically predicted the optimal ceRNA network based on MRE overlap and gene expression correlation. Interestingly, Gene Ontology analysis of this network revealed that numerous nodes are involved in regulation of gene expression (33). Furthermore, we predicted putative ceRNAs of the oncogenic transcription factor translocation PAX–FKHR and some of its transcriptional targets in rhabdomyosarcoma, and found that the presence of the PAX–FKHR chimeric transcription factor resulted not only in increases in its transcriptional targets but also in a higher abundance of some of its predicted ceRNAs and ceRNAs of its transcriptional targets (33). This indicates that transcription factors regulate both their targets and ceRNA networks linked to their targets, thus expanding the regulatory potency of transcription factors (Fig. 3B). This may have critical implications in cancers driven by genomic translocation that result in aberrant expression of

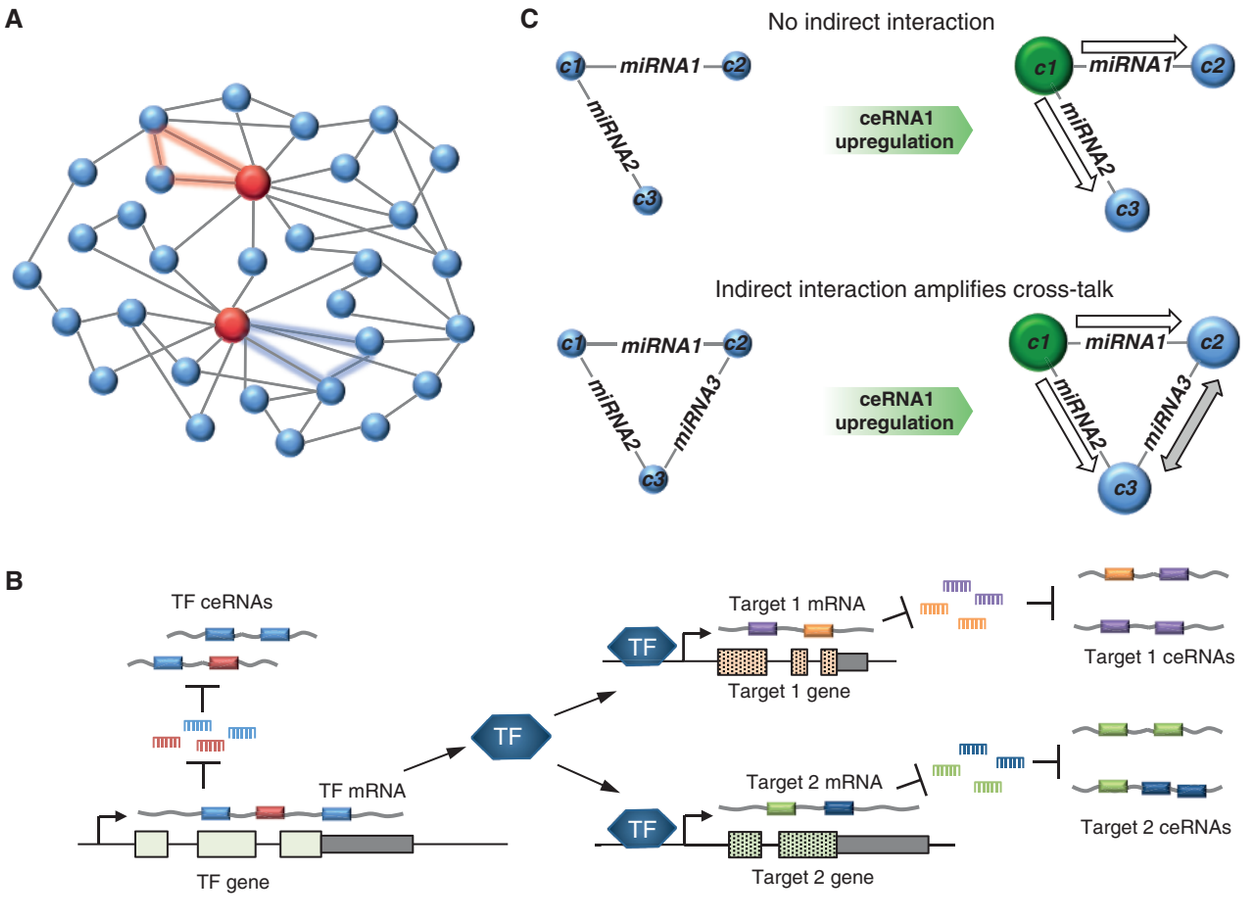

Figure 3. ceRNA networks. **A,** example of a ceRNET. The schematic depicts a ceRNET with a moderate degree of complexity that contains several indirect interactions (outlined by "glowing" connections). The nodes highlighted in red could represent critical nodes that are connected to numerous other ceRNAs and thus may have the most impact on the network when deregulated. **B,** intertwined transcriptional and ceRNA networks. A transcription factor may act as ceRNA via its mRNA transcript, whereas its protein stimulates transcription of targets that in turn also decoy miRNAs. TF, transcription factor. **C,** indirect interactions amplify ceRNA cross-talk. These two networks consisting of three ceRNAs differ only by a miRNA connection between ceRNA2 and ceRNA3. When this connection is lacking, ceRNA1 (shown in green) has only direct effects on ceRNAs 2 and 3 (top). If the connection between ceRNAs 2 and 3 is present, then increasing the abundance of ceRNA1 will have direct effects as well as indirect effects (bottom). In the latter case, elevated levels of ceRNA1 will increase ceRNA2, which in turn will increase ceRNA3. Moreover, ceRNA1 will affect ceRNA3, which will then affect ceRNA2, and thus ceRNAs2 and 3 are doubly regulated through ceRNA cross-talk.

(chimeric) transcription factors such as PAX–FKHR, ERG, and c-MYC.

Given the complexity of ceRNETs, future work will focus on identifying the critical nodes that have potent effects on the network or subnetworks when their expression levels are perturbed. Recently developed mathematical models will help to understand the complexity and behavior of ceRNETs (33, 56). These models were used to carry out relatively simple simulations involving only a handful of ceRNAs and miRNAs, but they are scalable to replicate larger networks. Moreover, although these networks currently allow only for qualitative predictions, a detailed knowledge of the identity and abundance of all components of a ceRNET will enable us to quantitatively simulate the behavior of a ceRNET in response to perturbation of a single factor. We combined the mathematical ceRNA model with cell biologic approaches in an attempt to further define the molecular conditions for optimal ceRNA cross-talk and made several interesting observations (33). First, *in silico* simulations showed that ceRNAs communicate best at near-equimolarity of all components involved in the cross-talk (i.e., ceRNAs and miRNAs). Second, the relative abundance of ceRNAs and miRNAs influences cross-talk, as does the number of MREs (i.e., the number of miRNAs) that two ceRNAs have in common. Third, and critically, indirect interactions where two linked ceRNAs are also connected through a third ceRNA greatly amplify ceRNA cross-talk (Fig. 3C). These results shed some light on the molecular environment that permits RNAs to serve as efficient miRNA decoys; however, more work is required to fully elucidate the mechanisms of ceRNA cross-talk.

CONCLUSION AND FUTURE CHALLENGES

The last few years have heralded the ceRNA hypothesis as an exciting novel aspect of RNA biology. Studies by several groups illustrate that ceRNAs serve as posttranscriptional

regulators of gene expression by decoying miRNAs from other target transcripts. Such sponge activity has been shown for various examples of mRNAs, pseudogenes, lncRNAs, and circRNAs, but virtually any transcript containing miRNA seed matches may participate in ceRNA cross-talk. Indeed, a recent study reported canonical and noncanonical miRNA-binding sites on numerous ribosomal RNAs and tRNAs (57). The ever-expanding variety of ceRNAs promises an exciting future where we will uncover the importance of newly discovered transcripts such as pseudogenes, lncRNAs, and circRNAs, and ascribe novel, protein coding–independent functions to well-studied mRNAs. The ceRNA field is certainly still in its infancy and several open questions remain to be addressed. For instance, what is the dynamic range of ceRNAs in tumorigenesis and normal physiology? A low-abundance ceRNA may have a potent effect when overexpressed several-fold, whereas its silencing may cause very little harm. But how low abundant is too low to have an effect? *PTENP1* seems to be expressed at lower levels than *PTEN* in some tissues, and yet its silencing has a significant effect on PTEN (13). Conversely, *VAPA* is more abundant than *PTEN* and has a much more profound effect on PTEN than vice versa (28, 33). Future work will need to address this issue. Why does the silencing of a single ceRNA have profound effects on another, even though the entire ceRNET should lend robustness and "dilute" the effect? Threshold levels, relative ceRNA ratios, miRNA affinity to seed matches, and cross-talk–amplifying indirect interactions may all contribute to the ceRNA effect (33), but this awaits experimental confirmation. How do RNA-binding proteins that could mask MREs, secondary structures of transcripts, RNA turnover, and subcellular compartmentalization influence ceRNA cross-talk? Many answers to these questions will be applicable only to a certain ceRNA or ceRNET, and possibly only in a specific tumor type—when the abundance or localization of ceRNET components changes, the efficiency of ceRNA cross-talk will likely change as well. Thus, each and every ceRNA will have to be evaluated individually and in the context and cell type of interest. Finally, the tumor-promoting capability of under- or overexpressed ceRNAs remains to be investigated in mouse models, and a relevance to human cancers needs to be firmly established. Even though numerous open questions remain, the discovery of endogenous miRNA decoys and ceRNETs could guide the functionalization of a sizeable portion of the cancer transcriptome, and may stimulate the development of novel and exciting cancer treatment strategies to target key nodes in ceRNETs that will add to existing approaches of miRNA therapy (58).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Writing, review, and/or revision of the manuscript: F.A. Karreth, P.P. Pandolfi

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