Molecular and Cellular Biochemistry (2021) 476:109–123
https://doi.org/10.1007/s11010-020-03889-2

The development and controversy of competitive endogenous RNA hypothesis in non-coding genes

Weimin Lin[1] · Hongcheng Liu[1] · Yonghang Tang[1] · Yuchen Wei[1] · Wei Wei[1] · Lifan Zhang[1] · Jie Chen[1]

Received: 3 May 2020 / Accepted: 14 August 2020 / Published online: 25 September 2020

Abstract
As a momentous post-transcriptional regulator, microRNAs (miRNAs) are attracting more and more attention. The classical miRNAs regulated mechanism shows it binds to the targets' 3′UTR thus play the role in post-transcription. Meanwhile, single miRNA can target multiple genes, so those should compete to bind that miRNA. Vice versa, single gene can sponge mass of miRNAs as well. Thus the competitive endogenous RNAs (ceRNAs) hypothesis was put forward in 2011. The ceRNA hypothesis has made huge achievements, in particular in non-coding genes, which including long non-coding RNAs (lncRNAs), circle RNAs (circRNAs) and pseudogenes, even viral transcripts. It also contributed greatly to epigenetics development. However, an increasing number of controversies have occurred with applause. Based on this situation, this review introduces something in detail about the ceRNAs hypothesis achieved in lncRNAs, circRNAs, pseudogenes and viral transcripts, respectively. Meanwhile, it also covers controversy of the ceRNAs hypothesis.

Keywords miRNA · ceRNA · lncRNA · circRNA · Pseudogene · Controversy

The classical miRNA biogenesis pathway

The first microRNA, lin-4, was found in nematode *Caenorhabditis elegans* before nearly four decades. Originally believed the *lin-4* was a protein coding gene; however, the product of *lin-4* unexpectedly was a 22-nucleotides regulatory RNA [1–3]. Since then, thousands of miRNAs have been found among kinds of species, including animals, plants, etc. Animal microRNAs are highly conserved among species. There are a mass of conserved and homologous miRNAs even in distinct species, which shows the biological functions of the miRNAs are crucial.

The processing of miRNAs contains the following parts: the first step is transcription of primary microRNAs, the second step is splicing of precursor microRNAs, and finally is miRNAs' maturing, as shown in Fig. 1.

First, RNA polymerase II (a few ones are RNA polymerase III) mediates the miRNA genes or introns produce a primary microRNA transcripts (pri-microRNA) with stem-loop structure, and whose length usually is thousands nucleotides [4, 5].

Subsequently, the endonuclease Drosha splices the pri-microRNA into precursor microRNA (pre-microRNA) with small hairpin structure, whose length approximately is 65 nucleotides [6–9]. This co-transcriptional mode raises widely consensus. It occurred during the primary transcripts that have not separated with the genome DNA yet. Then the pre-miRNAs are transported from nucleus to cytoplasm in Ran-GTPase-dependent manner by the exportin-5 [10–12]. Processing step occurred in the cytoplasm is essential for pre-miRNAs to become mature miRNAs. Stem-loop region is spliced by an endonuclease Dicer [13–15]. Duplex RNAs then via above two steps are produced, whose length generally is 22-nucleotides. Drosha and Dicer have the same RNase III splicing character thus lead to a 2 nucleotides overhang structure at 3′ end of duplex RNA. This structure is conducive to load into the RNA-induced silencing complex (RISC) [16]. It is worth to notice during Drosha and Dicer splice the precursor miRNAs, abundance of auxilins take part in the processing as a complex with above enzymes to make sure accurately splicing, take DGCR8 for Drosha [17], TRBP and PACT for Dicer [18, 19] for example. Argonaute proteins 2 (Ago2) is a key protein that incorporates duplex RNAs to become RISC [20]. Finally, one strand of

✉ Jie Chen
jiechen@njau.edu.cn

[1] Nanjing Agricultural University, Nanjing, China

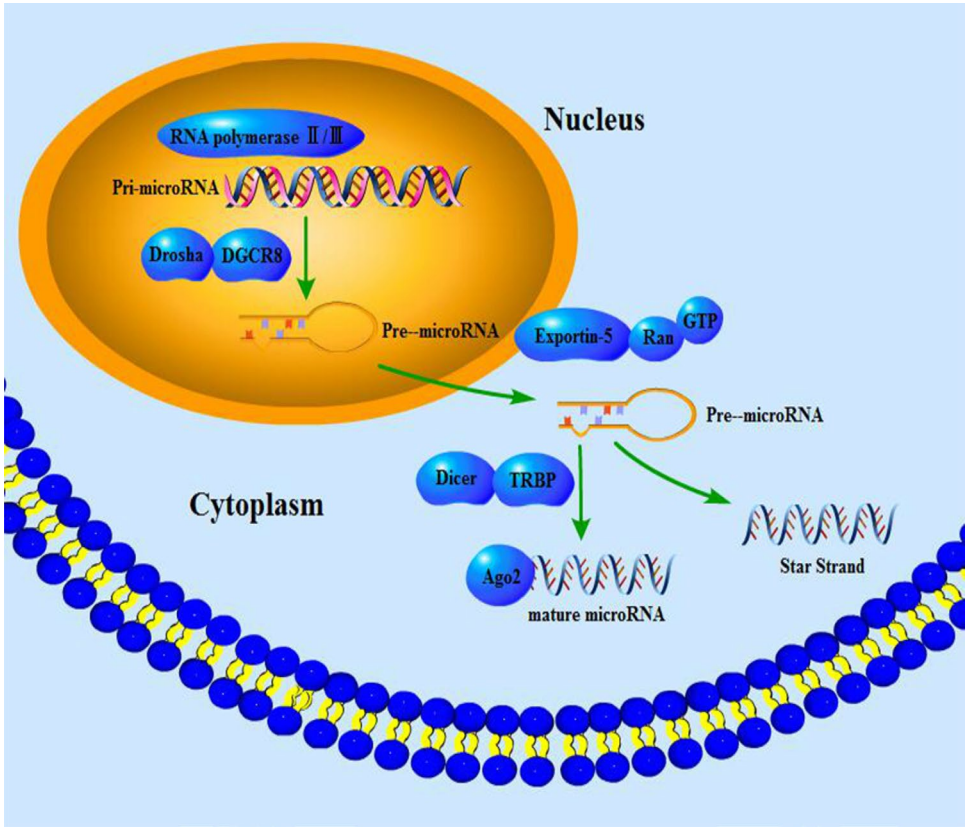

Fig.1 Canonical microRNA biogenesis pathway. In nucleus, the primary microRNAs are transcribed by RNA polymerase II, basically. Then, the enzyme Drosha splices the primary microRNAs to precursor microRNAs. The Exportin-5 transports the pre-microRNAs from nucleus to cytoplasm, with the offer in Ran-GTPase. Subsequently, Dicer finishes the last splicing program, which is key component to be a mature one. The microRNA finally loads into Ago2, which binds in the 3′UTR of target gene for post-transcriptional regulation

duplex loads into RISC as a mature miRNA, while another one named as star strand is typically degraded. However, for some miRNAs, both strands would load into RISC as mature miRNAs. Here, terms the strand from the 5′ end of the stem-loop as "5p", 3′ end as "3p" [21]. In fact, on account of next generation sequencing used extensively, what it has validated was a small fraction of star strand loaded for essentially all of miRNAs family [22]. Meanwhile, according to cell type or biological state, several miRNAs have different usages, thus lead to the nomenclature further complicated [23]. By contrast, the 5p/3p nomenclature is more reliable than stochastic mature/star nomenclature.

Development of the ceRNAs hypothesis

With the bioinformatics developed, the prediction targets of miRNAs become straightforward. As shown previously, mature miRNAs bind to target genes by complementary base pairing with nucleotides to play the post-transcriptional regulation, it locates on 3′UTR of coding gene, thus defines the nucleotides as microRNA response elements (MREs). While the binding sequence in miRNAs is as seed sequence, whose length usually is 6–8 nucleotides, it locates at 2–8 nt in 5′ end of miRNAs. Meanwhile, it is the size of MREs as well.

A miRNA can bind to abundant target genes containing same MREs, that is, target genes compete to sponge the miRNA. Thus, the competitive endogenous RNAs (ceRNAs) hypothesis is put forward.

The competitive endogenous RNAs (ceRNAs) hypothesis was firstly proposed by Pandolfi lab in journal CELL at 2011 and has received wide attention since then. They subsequently published an article to test the hypothesis. In the paper, the phosphatase and tensin homolog (*PTEN*) gene, which was known to be specifically abundant expressed during cancer developed, was utilized. Kinds of software were used to predict target genes which sponged the same miRNAs with *PTEN* (10 miRNAs have verified in published articles), and screened out the genes that sponged at least 6 miRNAs to perform subsequent functional verification. Since then, abundant of studies followed this study idea [24, 25].

In current, the research of ceRNAs effect covers a wide range of subjects. It involves not only in coding genes but many non-coding genes, including long non-coding RNAs (lncRNAs), circle RNAs (circRNAs), pseudogenes and viral transcripts. The lncRNAs, circRNAs and pseudogenes previously are deemed to the waste or noise of transcription. However, now we know those non-coding ones play an important post-transcriptional role.

Meanwhile, those non-coding genes also provide abundant research materials of ceRNA hypothesis, because they contain kinds of MREs, and usually around coding genes, for instance, lncRNAs and circRNAs. Moreover, a few miRNAs originates from some lncRNAs and circRNAs.

The lncRNAs as ceRNAs

In general, lncRNAs are regarded as by-products of the gene transcription process which lacked protein coding potential, and their size usually more than 200 nucleotides, even up to 1000 nucleotides. A few studies although demonstrated ncRNAs may engage ribosomes and produce small polypeptides, normally less than 100 amino acids [26]. However, the most reports suggested that lncRNAs did not encode proteins [27]. An accepted consensus of lncRNAs biological role is lncRNAs involves in epigenetic modulation in the nucleus, or as the post-transcriptional regulator in the cytoplasm via cis- or trans-regulation adjacent genes [28]. In the nucleus, several reports suggest lncRNAs directly interact with transcription factors as transcriptional co-activators, while others indicate lncRNAs may impair the assembly of transcriptional complexes, as the inhibitor of gene expression [29–31]. When lncRNAs in cytoplasm, they able to sponge the microRNAs, acts as the regulator to affect their target genes post-transcriptionally expressed. Thus, only the lncRNAs locate in the cytoplasm can act as ceRNAs, which is one such hypothesis for lncRNAs role to attract notable attention.

Huge studies demonstrate that the most lncRNAs are highly conserved in mammalian genomes, whether it is human or animals, which usually share a conserved region in the same lncRNA [32]. For ncRNAs are transcribed from genes, hence, the lncRNAs share the uniform MREs with genes, which is the key foothold for ceRNAs hypothesis. The lncRNAs are widely distributed in genome, according to GENCODE, which summarizes ENCODE project. There are 17,960 lncRNAs in human transcriptome and 13,197 in mouse transcriptome (Newly report of June 24th, 2020, shows in Fig. 2). Such abundant lncRNAs data provide the important materials to ceRNAs.

Numerous lncRNAs play a crucial regulatory role in human diseases occurred, which including Parkinson's disease [33], Cardiovascular Disease [34], Liver diseases [35], kinds of Cancer [36, 37], Obesity [38], and Muscular Atrophy [39].

Adipose tissue is the most important energy storage tissue of the body. Additionally, it also has the potential endocrine role, and secretes adipokines including leptin, adiponectin, resistin, interleukin, visfatin, etc. Meanwhile, it takes part in the immune and metabolic regulation. Adipose divides into white and brown adipose. The brown adipose tissue (BAT) offers chemical energy to the body by mobilizing more lipolysis of white adipose tissue (WAT), because of its ample mitochondrial content. The BAT mainly distributes in the interscapular area, armpit and back of neck. In particular, more BAT is contained in newborns and hibernating animals. However, some reports corroborate adult humans have metabolically active BAT [40, 41]. Recently studies demonstrate lncRNAs involve in the development and function of BAT. The lnc-BATE1 binds two protein heterogeneous nuclear ribonucleoprotein U necessary for BAT adipogenesis, and its inhibition impairs brown fat while activates the white fat-associated gene expression [42].The human brown fat lncRNA 1(Blnc1) generates the series of truncation mutants to identify the functional RNA domains, via RNA–protein interaction study to illuminate the molecular features of the Blnc1 ribonucleoprotein complex. Results show Blnc1 is highly conserved between human and mouse at both genomic and functional level, and it can facilitate the brown adipocyte-associated gene expression. Adiponectin antisense lncRNA can inhibit adipogenesis, including the adipogenesis of WAT, BAT and liver triglyceride (TG) by

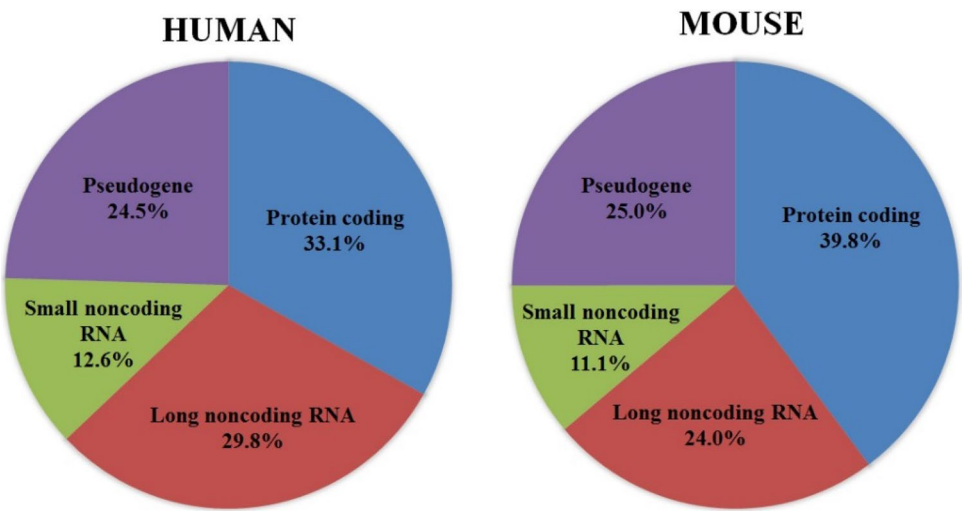

Fig2 Annotated transcripts in GENCODE. The pie charts show the current statistics on human and mouse transcripts identified in GENCODE. (Version34 and version M25, respectively, published in June 24th, 2020)

transferring it from nucleus to cytoplasm, and attenuate adiponectin mRNA translation at the same time [43].

Besides that, adipocyte differentiation-associated long non-coding RNA (ADNCR) is able to be a competitive endogenous RNA by sponging *miR-204* and therefore inhibits the bovine adipocyte differentiation [44]. The lncRNA Gm15290 promotes murine PPARγ-induced fat deposition by sponging *miR-27b*, as ceRNA of *PPARγ* [45]. A new lncRNA terms muscle differentiation-associated lncRNA (MDNCR) by sponging *miR-133a* to promote the bovine myoblast differentiation and inhibit cell proliferation, as ceRNA of *GosB* [46]. Meanwhile, a myogenesis-associated lncRNA (lncMG) acts as ceRNA for *IGF2* by sponging *miR-125b* to promote myogenesis [47]. The H19 is an important lncRNA, its exon1 encodes *miR-675-3p* and *miR-675-5p*, they target the *Smad1*, *Smad5* and *Cdc6* to regulate the skeletal muscle differentiation and regeneration. Meanwhile, H19 antagonizes the roles of these two miRNAs, acts as the ceRNAs of *β-catenin*, which is those miRNAs target gene *β-catenin* activates the Wnt/β-catenin pathway to promote the osteogenesis. In the other way, H19 as ceRNA of *C8orf4a* can sponge the *miR-30a* to modulate the adipogenic differentiation [48–50]. LncIRS1 as ceRNA of *IRS1* by sponging the *miR-15a* and *miR-15b/c-5p* to promote chicken skeletal muscle myogenesis and control atrophy. LncRNA MEG3 as ceRNA of *SRF* via sponging the *miR-423-5p* as ceRNA of *SRF*, lncRNA MEG3 inhibit myoblast proliferation and promote its differentiation [51]. To understand more effectively, Table 1 discusses about recent advancements of lncRNAs as ceRNAs.

The circRNAs as ceRNAs

The circRNAs are found over decades, while the mechanism of their biosynthesis is distinct. In current, the mostly accepted point is they are produced from precursor messenger RNA (pre-mRNA). During the mRNA transcription of exons, RNA is partially folded, thus leads exon skipping, which allows the region crossing to form circular RNA intermediates, and further back-splicing to be a circRNA. Another view suggests reverse complementarity leads introns complementary pairing, and excision of remaining introns and to form circRNA [52]. Since circRNA can derive from the gene transcript, different back-splicing modes and different back-splicing sites, one gene locus may produce multiple circRNAs [53]. According to the diverse characteristics of alternative back-splicing modes and splicing sites, they can be divided into 2 kinds of alternative back-splicing and 4 kinds of alternative splicing landscape. 2 kinds of alternative back-splicing (5′ back-splicing and 3′ back-splicing) and 4 kinds of alternative splicing (5′ splicing, alternative 3′ splicing, cassette exon and intron retention), respectively [54].

The most circRNAs locate in the cytoplasm, while a few circRNAs retaining intron sequences are often restricted to the nucleus [52, 55]. CircRNAs expression generally maintains at a low level. But there is abundant circRNAs expression in some tissues or cell types, such as in brain, and it is more abundant than linear expression [56, 57]. Besides, reports also suggest certain circRNAs are the origin of a few pseudogenes, for example, ring finger and WD repeat domain 2 circRNA (RFWD2) are the origin of over 30 pseudogenes [58].

The circRNA expression pattern is highly conserved in many species, the expression abundance in many tissues among individuals, particularly in mammal neuronal tissues. A striking example is 4522 out of total 15,849 mouse circRNAs are conserved in human brains, even some of them can be observed in fly brains [59]. The report corroborates same circRNAs are conserved in humans, mice, and flies, possibly because these ones have certain neurological functions [59]. The reason for the high expression and content in the nervous system is circRNAs are preferentially spliced. CircRNAs also act as the regulator in human-related diseases occurred, including various cancers. Research shows circRNAs relate to innate immune response, transfection of circRNAs derives from *vivo* into mammalian cells effectively induced immune genes expressed, thus enhances the protection against viral inflectional mammalian cells [60].

Furthermore, circRNAs are ability to sponge miRNAs act as ceRNAs of miRNAs targets and play post-transcriptional role. For example, CDR1 antisense RNA (CDR1as) is a cyclic, highly conserved and abundant single exon circRNA in mammalian brain, there are over 60 binding sites of *miR-7*. Besides, circular RNA sponge for *miR-7* (ciRS -7) is also abundant in human and mouse brains. CiRS-7 has more than 70 *miR-7* binding sites [61]. According to studies, CDR1as is able to regulate osteogenic differentiation of periodontal ligament stem cells via *miR-7*/*GDF5*/*SMAD* and p38/MAPK signaling pathway and CDR1as acts as ceRNA [62]. The mitochondrial fission and apoptosis-related circRNA (MFACR) mediates myocardial cell death by sponging *miR-552-3p* to upregulate mitochondrial fission process 1 gene (*MTP18*) expressed [63]. The itchy E3 ubiquitin protein ligase circRNA (circ-ITCH) regulates *p21* and *PTEN* genes' expression by sponging *miR-17* and *miR-224*, thereby inhibiting the bladder cancer developed [64]. ADP ribosylation factor 3 circRNA (circARF3) inhibits TNF receptor-associated factor 3 gene (*TRAF3*) by sponging *miR-103*, thus eases mitophagy-mediated inflammation in vitro and in vivo [65]. Homeodomain interacting protein kinase 3 circRNA (circHIPK3) sponges *miR-30a* to promote vascular endothelial growth factor C (*VEGF-C*), frizzled class receptor 4 (*FZD4*), Wnt family member 2 (*WNT2*) expressed, leads to endothelial cell proliferation and increases vascular dysfunction, blocks the *miR-30* a role in diabetic retinopathy

Table 1 Competing endogenous RNA studies

Type of ceRNAs	Example of ceRNAs	Key miRNA	Affected targets	Biological role	Compartments that ceRNA located	References
Long non-coding RNAs	ADNCR	*miR-204*	*SIRT1*	Inhibited adipocyte differentiation	Bovine Adipocyte-derived stem cells	[44]
	LncRNA Gm15290	*miR-27b*	*PPARγ*	Promoted PPARγ-induced fat deposition	Murine primary adipocytes	[45]
	MDNCR	*miR-133a*	*GosB*	Promoted myoblast differentiation and inhibited cell proliferation	Bovine myoblast cells	[46]
	Lncmg	*miR-125b*	*IGF2*	Promote myogenesis	Murine muscle stem cells	[47]
	H19	1.*miR-675-3p* 2.*miR-675-5p* 3.*miR-30a*	1/2.*Smad1*/*Smad5*/*Cdc6*/*β-catenin* 3. *C8orf4 a*	1/2.Regulate the skeletal muscle differentiation and regeneration 3. Modulates the adipogenic differentiation	1/2.C2C12 myoblast cell 3. human adipose tissue-derived mesenchymal stem cells	[46, 47, 50]
	MIAT	*miR-18a-5p*	*ESR1*	Regulated adipocyte differentiation	Human adipose-derived stem cells	[129]
	LINC02202	1.*miR-136-5p* 2.*miR-381-3p*	1.*PIK3R1* 2.*FOXO1*	Regulated adipocyte differentiation	Human adipose-derived stem cells	[129]
	LncRNA TINCR	miR-31-5p	*C/EBPα*	Modulates the adipogenic differentiation	Human adipose tissue-derived mesenchymal stem cells	[130]
	Lnc-231	*miR-125a-5p*	*E2F3*	Promoted myoblast proliferation and inhibited differentiation	Murine myoblast cells	[131]
	LncRNA MEG3	*miR-423-5p*	*SRF*	inhibited myoblast proliferation and promoted its differentiation	Porcine Satellite Cells	[51]
	LncIRS1	*miR-15a* *miR-15b-5p* *miR-15c-5p*	*IRS1*	Promoted skeletal muscle myogenesis and controlled atrophy	Chicken primary myoblasts	[132]
	RP11-142A22	*miR-587*	*Wnt5β*	Promoted adipogenesis	Human visceral adipose tissue	[133]
	LncRNA-Adi	*miR-449a*	*CDK6*, *CDC25A*	Regulated adipogenesis	Mouse adipose-derived stem cells	[134]

Table 1 (continued)

Type of ceRNAs	Example of ceRNAs	Key miRNA	Affected targets	Biological role	Compartments that ceRNA located	References
Circle RNAs	circHUWE1	*miR-29b*	*AKT3*	Regulated myoblast development	Bovine myoblast cells	[69]
	circSAMD4A	*miR-138-5p*	*EZH2*	Regulated preadipocyte differentiation	Human adipose tissue	[70]
	circErbB4	*miR-29a-5p*	*AT2R*	Induced vascular smooth muscle cell migration	Mouse aortic smooth muscle cells	[135]
	circHIPK3	*miR-326*	*STIM1*	Modulated airway smooth muscle cells proliferation	Human airway smooth muscle	[136]
	circINSR	*miR-34a*	*Bcl-2*, *CyclinE2*	Regulated myoblast cells proliferation and apoptosis	Bovine longissimus dorsi	[68]
	circTTN	*miR-432*	*IGF2*	Facilitated myoblasts proliferation and differentiation	Bovine primary myoblasts	[67]
	circTMTC1	*miR-128-3p*	*MSTN*	Inhibited chicken skeletal muscle satellite cell differentiation	Chicken skeletal muscle satellite cell	[137]
	circCDR1	*miR-7*	*IGF1R*	Induced myoblast differentiation	C2C12 myoblast cell	[138]
	circSNX29	*miR-744*	*Wnt5α*	Facilitated myoblasts differentiation and inhibited proliferation	C2C12 myoblast cell	[139]

Table 1 (continued)

Type of ceRNAs	Example of ceRNAs	Key miRNA	Affected targets	Biological role	Compartments that ceRNA located	References
Pseudogenes	PTENP1	1.*miR-21* 2.*miR-10a-5p* 3. *miR-214* 4.*miR-19b*	1/2/3.*PTEN* 4. *MTUS1*	1. Regulated smooth muscle cell proliferation and apoptosis; 2. Inhibited the glioma cell progression; 3. Modulated osteoclast differentiation and attenuated osteoporosis 4. Inhibited cell proliferation and invasion	1. Human aortic smooth muscle cells; 2. Human mesenchymal stem cells; 3. RAW 264.7 macrophages 4. Human cervical cancer cell lines	[140–143]
	BRAFP	*miR-30a* *miR-182* *miR-876* *miR-590*	*BRAF*	Modulated carcinogenesis	HCT116 cells and HeLa cells	[84]
	CYP4Z2P	*miR-204* *miR-211* *miR-125a-3p* *miR-197* *miR-1226*	*CYP4Z1*	Promoted breast cancer angiogenesis	Human breast cancer cell lines	[79, 80]
	HMGA1P6	*let-7c-5p, miR106a-5p miR-103a-3p*	*HMGA1*, *HMGA2*	Promoted ovarian cancer cell malignancy	HO-8910 ovarian cancer cell lines	[81]
	PDIA3P1	*miR-124-3p*	*RELA*	Promoted highly-invasive mesenchymal transition of glioma cells	Human glioma cell lines	[82]
	DUXAP8	*miR-577*	*RAB14*	Promoted colorectal cancer cell proliferation, migration and invasion, inhibited apoptosis	Human colorectal cancer cell lines	[83]
Viral transcripts	Influenza A Virus transcripts	*miR-101*	*mTOR*	Abrogated Viral Life Cycle	A549 cells	[144]
	hepatitis B Virus transcripts	*miR-15a/16*	*Bcl-2*, *Smad7*	Made cells resistant to apoptosis and promoted tumorigenesis	Human hepatoma cell lines	[97, 98]
	hepatitis C Virus transcripts	*miR-122*	*STAT3*	Repressed the cellular antiviral	Huh7 cells	[95]

[66]. In Qinchuan cattle, some circRNAs are identified as the ceRNA to regulate the myogenesis, for example, circTTN sponges *miR-432* to be ceRNA of insulin like growth factor 2 (*IGF2*) and facilitates myoblast proliferation and differentiation [67]. CircINSR acts as ceRNA of B cell leukemia/lymphoma 2 (*Bcl-2*) and Cyclin E 2 (*CyclinE2*) to regulate myoblast cells proliferation and apoptosis by sponging *miR-34a* [68]. The circHUWE1 acts as ceRNA of AKT serine/threonine kinase 3 (*AKT3*) to regulate myoblast development by sponging *miR-29b* [69]. Meanwhile, other reports demonstrate circSAMD4A acts as ceRNA of enhancer of zeste 2 polycomb repressive complex 2 subunit (*EZH2*) by sponging *miR-138-5p* to regulate preadipocyte differentiation in human adipose tissue [70]. To understand more effectively, Table 1 discusses about recent advancements of circRNAs as ceRNAs.

Pseudogene transcripts as ceRNAs

Pseudogenes, in the other word, are another type of long non-coding RNAs and generally consider as a subclass of lncRNAs. Pseudogenes and lncRNAs are very abundant in human and mouse genomes. According to the latest data of GENCODE, the content of pseudogenes in the published annotated information of human and mouse exceeds 24%, as shown in Fig. 3. Pseudogenes are very similar to the coding genes, because they are produced by modifying and cutting off the coding transcripts in the process of transcription. However, pseudogenes lost the ability to translate proteins, the reason is early appearance of termination codons in the sequence, or the occurrence of insertion or deletion of the shift-frames mutations, and usually denoted by ψ [71, 72]. On account of the high sequence homology between the pseudogenes and their parent genes, pseudogenes take part in post-transcriptional regulation of their parent genes. Mechanisms of regulation includes the formation of endogenous interfering RNAs, recruitment of regulatory proteins by pseudogene antisense RNAs to complementary sites in the parent genes to modulate chromatin remodeling, and competition for RNA-binding proteins or the translation machinery [73].

Besides, pseudogenes modulate parent genes' expression by competitive sponging the miRNAs shared between both as the ceRNA. The regulation is very obvious in the cancer that caused by cancer-specific pseudogenes abnormal expression [74]. Among them, pseudogene PTENP1 is the most well known and important one, of which the parent gene *PTEN* is a momentous label gene of carcinogenesis. Previous reports corroborated as the ceRNA, pseudogene PTENP1 modulated carcinogenesis by adjusting the expression of *PTEN* gene expressed [75, 76]. Pseudogene BRAFP acts as the ceRNA of B-Raf proto-oncogene,

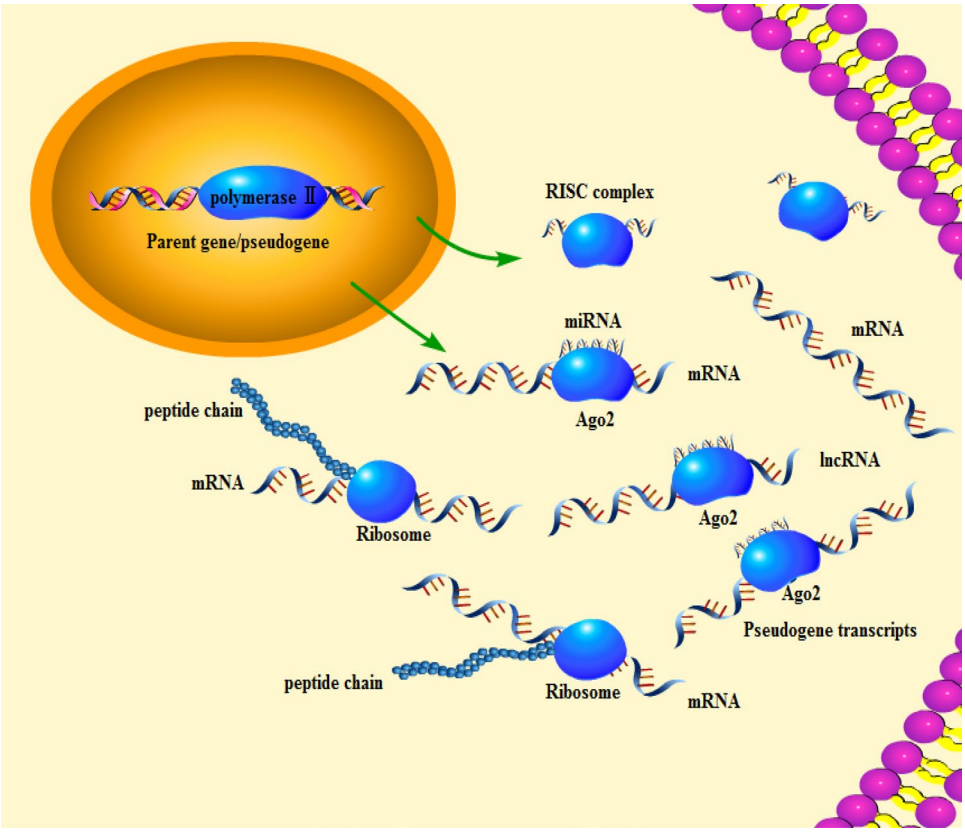

Fig.3 Mechanism of competitive endogenous RNA hypothesis. The miRNA is ubiquitous in cellular cytoplasm. In general, mRNA will translate to peptide chain, however, miRNA will negative regulate the translation of mRNA, which via binding on MREs. As shown in Fig. 1, mass of non-coding RNAs (lncRNAs, pseudogene transcripts and circRNAs, for example) also exist in cytoplasm. They have the identical MREs or binding sites like mRNA, thus those non-coding RNAs will sponge the miRNAs to relieve the miRNAs negative regulation to mRNA, and promote the expression of functional genes

serine/threonine kinase gene (*BRAF*) in humans and mouse to modulate carcinogenesis [73]. Pseudogene TUSC2P was highly homologous with its parent gene transcript 3′UTR, and the sequence of them can sponge multiple miRNAs (*miR-17*, *miR-93*, *miR-299-3p*, *miR-520a*, *miR-608* and *miR-661*). Pseudogene TUSC2P promotes tumor-suppressor 2 (*TUSC2)* expressed by competitive sponging those miRNAs to inhibit cell proliferation, survival, migration, invasion and colony formation, and increase tumor cell death [77]. Pseudogene HK2P1 competitive sponges *miR-6887-3p* to regulate hexokinase 2 gene (*HK2*) expressed, while reduces expression of HK2P1, *HK2* may contribute to the occurrence and development of preeclampsia by suppressing glycolysis and impairing decidualization [78]. Pseudogene CYP4Z2P by sponging miR-204, miR-211, miR-125a-3p, miR-197 and miR-1226 acts as ceRNA of *CYP4Z1* to promote breast cancer angiogenesis [79, 80]. Pseudogene HMGA1P6 acts as ceRNA of *HMGA1*and *HMGA2* by sponging let-7c-5p, miR-103a-3p and miR106a-5p to promote ovarian cancer cell malignancy [81]. Besides, pseudogene PDIA3P1 and DUXAP8 also, respectively, acts as ceRNA of *RELA* and *RAB14* to regulate glioma and colorectal cancer occurred [82, 83]. To understand more effectively, Table 1 discusses about recent advancements of pseudogenes as ceRNAs.

The viral transcripts acted as ceRNAs

The viruses infect host cells cause kinds of diseases, among which cancer and hepatitis are prominent ones. The mostly accepted points of pathogenesis are genomic message interaction existed between viruses and host cell, and the miRNAs is the key component of interaction [84–86]. Besides the cellular miRNAs involve in interaction of host-viral, the viruses that encode their own miRNAs are corroborated to cause the host cell silencing machinery [87, 88], thus leads to diseases. The first reported viral miRNAs originated from Epstein Barr virus (EBV) [89], followed by Kaposi's sarcoma-associated herpesvirus (KSHV) [90], β-herpesvirus human cytomegalovirus (HCMV) [91], human α-herpesvirus herpes simplex virus-1 (HSV-1) [88], and heliothis virescens ascovirus (HvAV) [92]. As shown previously, miRNAs originated from viruses and cells involve in diseases. Among them, *miR-122* is prominent who extensively exists in liver. Kinds of hepatitis virus exhibit special tropism to the liver, so the *miRNA-122* is the indispensable factor of them to cause the diseases [93, 94]. For example, hepatitis C Virus (HCV) transcripts act as ceRNA of signal transducer and activator of transcription 3 (*STAT3*) gene to repress cellular antiviral in liver [95].

Currently, viral transcripts have demonstrated that acted as the ceRNAs to play the prominent role in diseases occurred. A viral transcript derives from herpesvirus saimiri named as U-rich non-coding RNAs of unknown function (HSURs) sponges three host cell miRNAs, take *miR-27* for example, one of them, binds to HSURs as ceRNAs of its target gene and leads to decrease its availability [96]. Meanwhile, Hepatitis B virus (HBC), its mRNA sponges *miR-15a* as the ceRNA of *Smad7* gene to involve in the TGF-β pathway; Smad7 is the key regulator of TGF-β, and it inhibits the TGF-β-induced apoptosis while facilitates tumorigenesis [97, 98]. However, it is still limited to the achievement on viral transcripts acting as ceNRAs.

The controversy of ceRNAs hypothesis

The proposal of the competitive endogenous RNA hypothesis provides a new perspective for the study of post-transcriptional regulation of genes, which was verified by a large number of researches. However, with the hypothesis developed, correspondingly emerges some new views challenge the ceRNAs hypothesis developed. The main of controversy focus on the following:

First, according to ceRNAs hypothesis, the abundance of individual target gene can modulate the activity of miRNAs. However, the expression alteration of an individual gene can constitute only a tiny fraction of miRNAs' target gene abundance. Thus some believe there should be a sensitive threshold for miRNA function, which leads miRNA to cause the ceRNAs effect [99–101]. When it is below the sensitive threshold, the influence of ceRNAs effect is difficult to be observed. Meanwhile, for mRNAs, it requires extra high expression above the normal physiological level to compare with those of artificial miRNA sponges [102]. In general, a typical mRNA contains 1 or 2 binding sites of single miRNA, may express 10–100 copies per cell, while the level of miRNAs for various types of cells are estimated in ten to more than ten thousand copies per cell; thus it is impossible to decrease miRNA expressed significantly and make an effect on other mRNAs [103–106].

On the other hand, the binding energy is another point of ceRNAs hypothesis controversy. The binding site or MERs of miRNAs contains three kinds of size, ~ 6nt, ~ 7nt and ~ 8nt, respectively. The binding energy of miRNAs whether correlates with the length of binding site still is controversial. There is hierarchy for miRNAs bind to MERs or binding sites. In general, miRNAs preferentially bind to high affinity ~ 8nt and ~ 7nt, and then to ~ 6nt [101, 103, 107]. However, higher abundance of size is ~ 6nt, while lower abundance is ~ 7nt and ~ 8nt in vivo [108, 109]. Besides the miRNAs seed sequence complementary pairing with MERs of target genes, series of miRNAs contain supplementary base region that complementary pairing with sequence of target genes, which near to seed sequence. The seed sequence usually locates at 2–8 nucleotides of 5′ end of miRNAs while the supplementary region generally locates

at 12–16 nucleotides. There is a view that the supplementary region can elevate seed-match target genes recognized [110–112].

The canonical regulation of miRNAs in vivo as shown previously is loaded into argonaute protein (Ago2 in general) as a complex, thus the abundance of argonaute is another limit factor for ceRNAs cross-talk. The report demonstrates gene expression is altered by the competition of small RNAs, including miRNAs, the intermediate range level of argonaute promotes the competition, and the lower level of argonaute facilitates the stronger competition [113]. Besides, another report demonstrates Ago2 mRNA m6A methylation can modulate miRNA abundance thus to affect the ceRNA effect [114]. Here, whose involves m6A methylation, thus with various regulatory modes intervened, leads miRNA to play ceRNA effect in vivo became more complex.

The ceRNAs hypothesis bases on the canonical model of miRNAs binding to target genes, but many researches validate 60% of miRNA binding activity is non-canonical. To be specific, other parts of miRNAs escape the binding of seed sequence or with seed-like motifs, including mismatches or bulges take part in binding [115, 116]. Moreover, the RNA editing able to create or destroy the miRNA binding sites in 3′UTR of targets, which influences the miRNA binding activity, thus leads to modulate the ceRNA effect. Among them, Adenosine-to-inosine (A-to-I) editing is the most abundant modes in mammal, which modulates the miRNA binding sites in 3′UTR of targets [117]. This undoubtedly is another important factor affects the abundance of MREs.

Furthermore, series of non-coding genes are brought into focus. In general, even the abundance of lncRNAs is identified in particular tissues or organ, take skeletal muscle, fat, and brain for example. However the mostly individual lncRNA, circRNA and pseudogene expressed are far from the expression level of their corresponding ceRNAs (those ones usually are coding genes). In vitro experiments, however, artificially boost the expression of non-coding genes in excess. For example, the steady-state expression level of pseudogenes rarely reached that of their parent genes. In experimental models, parent genes and pseudogenes expression levels generally are comparable to demonstrate competitiveness, which differs from the real in vivo [118]. Meanwhile, the controversy over the hypothesis is further tanglesome by asserting that ceRNAs activity is a general phenomenon, thus ceRNAs are easy to define as a mechanism regulatory function class, in which linRNAs, circRNAs and pseudogenes totally contained [118, 119].

Second, the single source of non-coding genes. In the experimental model, the non-coding genes are usually focused on, which by the virtue of their derivation from reverse transcription of the same mRNA. Here, the non-coding genes originate very onefold, far less abundant than the non-coding genes produced in vivo. The experimental model studies the role of single non-coding gene only, while ignores the interaction among different non-coding genes. However, there is no reasonable method to solve that at present.

Third, point of controversy is the false-positive prediction of software. Currently, via online prediction websites or software to predict: for example, TargetScan [120], miRanda [121], RNA22 [122], PicTar [123], and PITA [124]. Nonetheless, target genes predict miRNAs or on the contrary, the false positive is inescapable. No matter which database is used, the principle is basically within uniform way, which is to estimate whether has miRNAs "seed sequence" (nucleotides 2–8 nt at the 5′ end) complimentary to MREs. However, the structure of numerous genes has not verified in practice yet, and there are practical physiological constraints and the interaction in vivo. Thus, many false-positive results will inevitably occur in the predicted results. To avoid false positive, the comprehensive utilization of multiple prediction software is helpful to obtain the intersection target genes. In addition, the interference of false positive can also be reduced using multiple miRNAs that has verified. Nevertheless, these methods are time-consuming and laborious, and still unable to completely avoid false positive.

Besides that, another key component is the balance of transcription and degradation between miRNAs and targets, in general, miRNAs-Ago2 complex binds and unbinds to targets is much faster than RNA transcription and degradation. Note that, transcription and degradation times of average length mRNA (miRNAs targets) have illustrated more 10 times than miRNA-Ago2 complex binds and unbinds to targets. The transcription and degradation are about 100 min, while the binding after 10 min expected to unbind [125, 126]. In the other words, if the complex unbinds from targets, and it is free and recycle binds to targets, so the abundance of miRNAs is the key regulator during ceRNAs effect. However, if complex unable to free and recycle, the ceRNAs effect will suffer huge controversy. Lots of researches have noticed the phenomenon that half-life difference between miRNAs and miRNA-depend targets. Among them, numerous miRNAs ceRNAs interaction net is another influence factor. General speaking, co-regulation between miRNAs and miRNA-depend targets is a dynamic course. To fuse the interaction among them, kinetics model maybe is worthy of consideration. In this model, common enzyme kinetics parameters K_{on}, K_{off}, K_{cat}, and K_{m} can build the kinetics model after mathematical derivation. Though the actual interaction in vivo can not be detected by experimental methods, however, the model can help us understand this dynamic course, thereby to explore ceRNAs effect in interaction of multiple miRNAs and miRNA-depend targets in vivo.

All the same, the ceRNA hypothesis still provides a new perspective for epigenetics development, and competitive

regulation has a significant influence. To intuitively understand, Fig. 3 discusses about ceRNAs effect in vivo.

After all, there are many competitive regulations in biological processes. New examples of RNA competition are found, including the inhibition of miRNAs in the process of transposition from replacement of RNA-binding protein s145 to RNA competition [127, 128]. It will further promote epigenetics development.

Acknowledgements This work was supported by the National Natural Science Foundation of China (Grant numbers 31872334, 31902132).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Horvitz H, Sulston J (1980) Isolation and genetic characterization of cell-lineage mutants of the nematode *Caenorhabditis elegans*. Genetics 96(2):435–454
2. Wightman B, Ha I, Ruvkun G (1993) Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. Cell 75(5):855–862
3. Lee R, Feinbaum R, Ambros V (1993) The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75(5):843–854. https://doi.org/10.1016/0092-8674(93)90529-y
4. Lee Y, Kim M, Han J, Yeom K, Lee S, Baek S, Kim V (2004) MicroRNA genes are transcribed by RNA polymerase II. EMBO J 23(20):4051–4060
5. Borchert G, Lanier W, Davidson B (2006) RNA polymerase III transcribes human microRNAs. Nat Struct Mol Biol 13(12):1097–1101
6. Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Rådmark O, Kim S, Kim V (2003) The nuclear RNase III Drosha initiates microRNA processing. Nature 425(6956):415–419
7. Morlando M, Ballarino M, Gromak N, Pagano F, Bozzoni I, Proudfoot N (2008) Primary microRNA transcripts are processed co-transcriptionally. Nat Struct Mol Biol 15(9):902–909
8. Han J, Lee Y, Yeom K, Kim Y, Jin H, Kim V (2004) The Drosha-DGCR8 complex in primary microRNA processing. Genes Dev 18(24):3016–3027
9. Denli A, Tops B, Plasterk R, Ketting R, Hannon G (2004) Processing of primary microRNAs by the Microprocessor complex. Nature 432(7014):231–235
10. Yi R, Qin Y, Macara I, Cullen B (2003) Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev 17(24):3011–3016
11. Bohnsack M, Czaplinski K, Gorlich D (2004) Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA 10(2):185–191
12. Lund E, Güttinger S, Calado A, Dahlberg J, Kutay U (2004) Nuclear export of microRNA precursors. Science 303(5654):95–98
13. Ketting RF, Fischer SE, Bernstein E, Sijen T, Hannon GJ, Plasterk RH (2001) Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in *C. elegans*. Genes Dev 15(20):2654–2659
14. Bernstein E, Caudy A, Hammond S, Hannon G (2001) Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature 409(6818):363–366
15. Hutvágner G, McLachlan J, Pasquinelli A, Bálint E, Tuschl T, Zamore P (2001) A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science 293(5531):834–838
16. He L, He X, Lim L, de Stanchina E, Xuan Z, Liang Y, Xue W, Zender L, Magnus J, Ridzon D, Jackson A, Linsley P, Chen C, Lowe S, Cleary M, Hannon G (2007) A microRNA component of the p53 tumour suppressor network. Nature 447(7148):1130–1134. https://doi.org/10.1038/nature05939
17. O'Donnell K, Wentzel E, Zeller K, Dang C, Mendell J (2005) c-Myc-regulated microRNAs modulate E2F1 expression. Nature 435(7043):839–843
18. Pradhan M, Prasad N, Palakal M (2012) A systems biology approach to the global analysis of transcription factors in colorectal cancer. BMC cancer 12:331
19. Shaulian E, Karin M (2002) AP-1 as a regulator of cell life and death. Nat Cell Biol 4(5):E131–136
20. Eferl R, Wagner E (2003) AP-1: a double-edged sword in tumorigenesis. Nat Rev Cancer 3(11):859–868. https://doi.org/10.1038/nrc1209
21. Hammond S (2015) An overview of microRNAs. Adv Drug Deliv Rev 87:3–14. https://doi.org/10.1016/j.addr.2015.05.001
22. Jr-Shiuan Y, Phillips MD, Doron B, Ping M, Andrea V, Siepel AC, Chen KC, Lai EC (2011) Widespread regulatory activity of vertebrate microRNA* species. RNA 17(2):312–326
23. Ohanian M, Humphreys D, Anderson E, Preiss T, Fatkin D (2013) A heterozygous variant in the human cardiac miR-133 gene, MIR133A2, alters miRNA duplex processing and strand abundance. BMC Genet 14:18
24. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP (2011) ceRNA hypothesis: the rosetta stone of a hidden RNA language? Cell 146(3):353–358
25. Tay Y, Kats L, Salmena L, Weiss D, Tan SM, Ala U, Karreth F, Poliseno L, Provero P, Dicunto F (2011) Coding-independent regulation of the tumor suppressor PTEN by competing endogenous mRNAs. Cell 147(2):344–357
26. Ingolia NT, Lareau LF, Weissman JS (2011) Ribosome profiling of mouse embryonic stem cells reveals the complexity and dynamics of mammalian proteomes. Cell 147(4):789–802
27. Mitchell G, Pamela R, Ingolia NT, Weissman JS, Lander ES (2013) Ribosome profiling provides evidence that large noncoding RNAs do not encode proteins. Cell 154(1):240–251
28. Sun M, Kraus WL (2015) From discovery to function: the expanding roles of long noncoding RNAs in physiology and disease. Endocr Rev 36(1):25–64
29. Jianchi F, Chunming B, Clark BS, Rina M, Palak S, Kohtz JD (2006) The Evf-2 noncoding RNA is transcribed from the Dlx-5/6 ultraconserved region and functions as a Dlx-2 transcriptional coactivator. Genes Dev 20(11):1470–1484
30. Igor M, Aroul R, Ana SB, Natalie C, Alexandre A (2007) Repression of the human dihydrofolate reductase gene by a non-coding interfering transcript. Nature 445(7128):666–670
31. Zhao XY, Li S, Wang GX, Yu Q, Lin J (2014) A long noncoding RNA transcriptional regulatory circuit drives thermogenic adipocyte differentiation. Mol Cell 55(3):372–382
32. Tichon A, Gil N, Lubelsky Y, Havkin ST, Lemze D, Itzkovitz S, Stern-Ginossar N, Ulitsky I (2016) A conserved abundant cytoplasmic long noncoding RNA modulates repression by Pumilio proteins in human cells. Nat Commun 7:12209
33. Kraus TFJ, Haider M, Spanner J, Steinmaurer M, Dietinger V, Kretzschmar HA (2016) Altered long noncoding rna expression precedes the course of Parkinson's disease—a preliminary report. Mol Neurobiol 54(4):2869–2877

34. Sallam T, Sandhu J, Tontonoz P (2018) Long noncoding RNA discovery in cardiovascular disease: decoding form to function. Circ Res 122(1):155
35. Kenji T, Irene Y, Hiroaki H, Tushar P (2014) Long noncoding RNA in liver diseases. Hepatology 60(2):744–753
36. Martens-Uzunova ES, Böttcher R, Croce CM, Jenster G, Visakorpi T, Calin GA (2014) Long noncoding RNA in prostate, bladder, and kidney cancer. Eur Urol 65(6):1140–1151
37. Lin C, Yang L (2018) Long noncoding RNA in cancer: wiring signaling circuitry. Trends Cell Biol 28(4):287–301. https://doi.org/10.1016/j.tcb.2017.11.008
38. Wei S, Du M, Jiang Z, Hausman G, Zhang L, Dodson M (2016) Long noncoding RNAs in regulating adipogenesis: new RNAs shed lights on obesity. Cell Mol Life Sci 73(10):1–9
39. Kramer NJ, Gitler AD (2017) Raise the roof: boosting the efficacy of a spinal muscular atrophy therapy. Neuron 93(1):3
40. Jan N, Tore B, Barbara C (2007) Unexpected evidence for active brown adipose tissue in adult humans. Am J Physiol 293(2):444–452
41. van Lans AA, Vosselman MJ, Hanssen MJW, Brans B, Lichtenbelt WDVM (2016) Supraclavicular skin temperature and BAT activity in lean healthy adults. J Physiol Sci 66(1):77–83
42. Alvarez-Dominguez J, Bai Z, Xu D, Yuan B, Lo K, Yoon M, Lim Y, Knoll M, Slavov N, Chen S, Peng C, Lodish H, Sun L (2015) De novo reconstruction of adipose tissue transcriptomes reveals long non-coding RNA regulators of brown adipocyte development. Cell Metab 21(5):764–776. https://doi.org/10.1016/j.cmet.2015.04.003
43. Cai R, Sun Y, Qimuge N, Wang G, Wang Y, Chu G, Yu T, Yang G, Pang W (2018) Adiponectin AS lncRNA inhibits adipogenesis by transferring from nucleus to cytoplasm and attenuating Adiponectin mRNA translation. Biochem Biophys Acta 4:420
44. Li M, Sun X, Cai H, Sun Y, Plath M, Li C, Lan X, Lei C, Lin F, Bai Y (2016) Long non-coding RNA ADNCR suppresses adipogenic differentiation by targeting miR-204. Biochem Biophys Acta 7:871–882
45. Liu W, Ma C, Yang B, Yin C, Zhang B, Xiao Y (2017) LncRNA Gm15290 sponges miR-27b to promote PPARÎ³-induced fat deposition and contribute to body weight gain in mice. Biochem Biophys Res Commun 493(3):1168
46. Li H, Yang J, Jiang R, Wei X, Song C, Huang Y, Lan X, Lei C, Ma Y, Hu L (2018) Long non-coding RNA profiling reveals an abundant MDNCR that promotes differentiation of myoblasts by sponging miR-133a. Mol Ther Nucleic Acids 12:610–625
47. Zhu M, Liu J, Xiao J, Yang L, Cai M, Shen H, Chen X, Ma Y, Hu S, Wang Z (2017) Lnc-mg is a long non-coding RNA that promotes myogenesis. Nat Commun 8:14718
48. Dey BK, Karl P, Anindya D (2014) The H19 long noncoding RNA gives rise to microRNAs miR-675-3p and miR-675-5p to promote skeletal muscle differentiation and regeneration. Genes Dev 28(5):491–501
49. Liang WC, Fu WM, Wang YB, Sun YX, Xu LL, Wong CW, Chan KM, Li G, Waye MY, Zhang JF (2016) H19 activates Wnt signaling and promotes osteoblast differentiation by functioning as a competing endogenous RNA. Sci Rep 6:20121
50. Li K, Wu Y, Yang H, Hong P, Fang X, Hu Y (2019) H19/miR-30a/C8orf4 axis modulates the adipogenic differentiation process in human adipose tissue-derived mesenchymal stem cells. J Cell Physiol 234(11):20925–20934. https://doi.org/10.1002/jcp.28697
51. Cheng X, Li L, Shi G, Chen L, Fang C, Li M, Li C (2020) MEG3 promotes differentiation of porcine satellite cells by sponging miR-423–5p to relieve inhibiting effect on SRF. Cells 9(2):449. https://doi.org/10.3390/cells9020449
52. Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, Marzluff WF, Sharpless NE (2013) Circular RNAs are abundant, conserved, and associated with ALU repeats. RNA 19(2):141–157
53. Gao Y, Wang J, Zheng Y, Zhang J, Chen S, Zhao F (2016) Comprehensive identification of internal structure and alternative splicing events in circular RNAs. Nat Commun 7:12060
54. Zhang XO, Dong R, Zhang Y, Zhang JL, Luo Z, Zhang J, Chen LL, Yang L (2016) Diverse alternative back-splicing and alternative splicing landscape of circular RNAs. Genome Res 26(9):1277–1287
55. Hall I, Climent M, Quintavalle M, Farina F, Schorn T, Zani S, Carullo P, Kunderfranco P, Civilini E, Condorelli G, Elia L (2019) Circ_Lrp6, a circular RNA enriched in vascular smooth muscle cells, acts as a sponge regulating miRNA-145 function. Circ Res 124(4):498–510. https://doi.org/10.1161/circresaha.118.314240
56. Zhang XO, Wang HB, Zhang Y, Lu X, Chen LL, Yang L (2014) Complementary sequence-mediated exon circularization. Cell 159(1):134–147
57. Xintian Y, Irena V, Ana B, Tristan W, Irina E, Georgi T, Güney A, Mantian W, Caspar G, Claudia Q (2015) Neural circular RNAs are derived from synaptic genes and regulated by development and plasticity. Nat Neurosci 18(4):603–610
58. Dong R, Zhang XO, Zhang Y, Ma XK, Chen LL, Yang L (2016) CircRNA-derived pseudogenes. Cell Res 26(6):747–750
59. Agnieszka RW, Christin S, Petar GA, Marvin J, Natalia P, Sebastian G, Mor H, Mikaela B, Osnat B, Reut AF (2015) Circular RNAs in the mammalian brain are highly abundant, conserved, and dynamically expressed. Mol Cell 58(5):870–885
60. Chen YG, Kim MV, Chen X, Batista PJ, Aoyama S, Wilusz JE, Iwasaki A, Chang HY (2017) Sensing self and foreign circular RNAs by intron identity. Mol Cell 67(2):S1097276517303623
61. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Bente F, Damgaard CK, Kjems J (2013) Natural RNA circles function as efficient microRNA sponges. Nature 495(7441):384–388
62. Li X, Zheng Y, Zheng Y, Huang Y, Zhang Y, Jia L, Li W (2018) Circular RNA CDR1as regulates osteoblastic differentiation of periodontal ligament stem cells via the miR-7/GDF5/SMAD and p38 MAPK signaling pathway. Stem Cell Res Ther 9(1):232
63. Wang K, Gan TY, Li N, Liu CY, Zhou LY, Gao JN, Chen C, Yan KW, Ponnusamy M, Zhang YH (2017) Circular RNA mediates cardiomyocyte death via miRNA-dependent upregulation of MTP18 expression. Cell Death Differ 24(6):1111–1120
64. Yang C, Yuan W, Yang X, Li P, Wang J, Han J, Tao J, Li P, Yang H, Lv Q (2018) Circular RNA circ-ITCH inhibits bladder cancer progression by sponging miR-17/miR-224 and regulating p21. PTEN Expr Mol Cancer 17(1):19
65. Zhang Z, Zhang T, Feng R, Huang H, Xia T, Sun C (2018) circARF3 alleviates mitophagy-mediated inflammation by targeting miR-103/TRAF3 in mouse adipose tissue. Mol Ther-Nucleic Aicds 14(12):192–203
66. Shan K, Liu C, Liu BH, Chen X, Dong R, Liu X, Zhang YY, Liu B, Zhang SJ, Wang JJ (2017) Circular non-coding RNA HIPK3 mediates retinal vascular dysfunction in diabetes mellitus. Circulation 136(17):1629
67. Wang X, Cao X, Dong D, Shen X, Cheng J, Jiang R, Yang Z, Peng S, Huang Y, Lan X, Elnour I, Lei C, Chen H (2019) Circular RNA TTN acts as a miR-432 sponge to facilitate proliferation and differentiation of myoblasts via the IGF2/PI3K/AKT signaling pathway. Mol Ther Nucleic acids 18:966–980. https://doi.org/10.1016/j.omtn.2019.10.019
68. Shen X, Zhang X, Ru W, Huang Y, Lan X, Lei C, Chen H (2020) circINSR promotes proliferation and reduces apoptosis of embryonic myoblasts by sponging miR-34a. Mol Ther Nucleic Acids 19:986–999. https://doi.org/10.1016/j.omtn.2019.12.032
69. Yue B, Wang J, Ru W, Wu J, Cao X, Yang H, Huang Y, Lan X, Lei C, Huang B, Chen H (2020) The circular RNA circHUWE1

sponges the miR-29b-AKT3 axis to regulate myoblast development. Mol Ther Nucleic acids 19:1086–1097. https://doi.org/10.1016/j.omtn.2019.12.039
70. Liu Y, Liu H, Li Y, Mao R, Yang H, Zhang Y, Zhang Y, Guo P, Zhan D, Zhang T (2020) Circular RNA SAMD4A controls adipogenesis in obesity through the miR-138-5p/EZH2 axis. Theranostics 10(10):4705–4719. https://doi.org/10.7150/thno.42417
71. Goodhead I, Darby AC (2015) Taking the pseudo out of pseudogenes. Curr Opin Microbiol 23:102–109
72. Poliseno L (2012) Pseudogenes: newly discovered players in human cancer. Sci Signal 5(242):re5. https://doi.org/10.1126/scisignal.2002858
73. Karreth F, Reschke M, Ruocco A, Ng C, Chapuy B, Léopold V, Sjoberg M, Keane T, Verma A, Ala U (2015) The BRAF pseudogene functions as a competitive endogenous RNA and induces lymphoma invivo. Cell 161(2):319–332
74. Kalyana-Sundaram S, Kumar-Sinha C, Shankar S, Robinson D, Wu YM, Cao X, Asangani I, Kothari V, Prensner J, Lonigro R (2012) Expressed pseudogenes in the transcriptional landscape of human cancers. Cell 149(7):1622–1634
75. Poliseno L, Pandolfi PP (2015) PTEN ceRNA networks in human cancer. Methods 77–78:41–50
76. Poliseno L, Salmena L, Zhang J, Carver B, Haveman W, Pandolfi P (2010) A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. Nature 465(7301):1033–1038. https://doi.org/10.1038/nature09144
77. Rutnam ZJ, Du WW, Yang W, Yang X, Yang BB (2014) The pseudogene TUSC2P promotes TUSC2 function by binding multiple microRNAs. Nat Commun 5(1):2914
78. Lv H, Tong J, Yang J, Lv S, Li W, Zhang C, Chen Z (2018) HK2P1Dysregulated pseudogene may contribute to preeclampsia as a competing endogenous RNA for hexokinase 2 by impairing decidualization. Hypertension 71(4):648–658. https://doi.org/10.1161/hypertensionaha.117.10084
79. Lufeng Z, Xiaoman L, Yi G, Xiaobo L, Tao X (2015) The 3'UTR of the pseudogene CYP4Z2P promotes tumor angiogenesis in breast cancer by acting as a ceRNA for CYP4Z1. Breast Cancer Res Treat 150(1):105–118
80. Zheng L, Li X, Gu Y, Lv X, Xi T (2020) Correction to: The 3'UTR of the pseudogene CYP4Z2P promotes tumor angiogenesis in breast cancer by acting as a ceRNA for CYP4Z1. Breast Cancer Res Treat 179(2):521–522. https://doi.org/10.1007/s10549-019-05478-4
81. Tian X, Song J, Zhang X, Yan M, Wang S, Wang Y, Xu L, Zhao L, Wei J, Shao C, Kong B, Liu Z (2020) MYC-regulated pseudogene HMGA1P6 promotes ovarian cancer malignancy via augmenting the oncogenic HMGA1/2. Cell Death Dis 11(3):167. https://doi.org/10.1038/s41419-020-2356-9
82. Wang S, Qi Y, Gao X, Qiu W, Liu Q, Guo X, Qian M, Chen Z, Zhang Z, Wang H, Xu J, Xue H, Guo X, Zhang P, Zhao R, Li G (2020) Hypoxia-induced lncRNA PDIA3P1 promotes mesenchymal transition via sponging of miR-124-3p in glioma. Cell Death Dis 11(3):168. https://doi.org/10.1038/s41419-020-2345-z
83. Du C, Wang H, Chen P, Chen C (2019) STAT3-induced upregulation of lncRNA DUXAP8 functions as ceRNA for miR-577 to promote the migration and invasion in colorectal cancer through the regulation of RAB14. Eur Rev Med Pharmacol Sci 23(14):6105–6118. https://doi.org/10.26355/eurrev_201907_18424
84. Gottwein E, Cullen B (2008) Viral and cellular microRNAs as determinants of viral pathogenesis and immunity. Cell Host Microbe 3(6):375–387
85. Bogerd H, Skalsky R, Kennedy E, Furuse Y, Whisnant A, Flores O, Schultz K, Putnam N, Barrows N, Sherry B, Scholle F, Garcia-Blanco M, Griffin D, Cullen B (2014) Replication of many human viruses is refractory to inhibition by endogenous cellular microRNAs. J Virol 88(14):8065–8076
86. Ghosh Z, Mallick B, Chakrabarti J (2009) Cellular versus viral microRNAs in host-virus interaction. Nucleic Acids Res 37(4):1035–1048. https://doi.org/10.1093/nar/gkn1004
87. Dunn W, Trang P, Zhong Q, Yang E, van Belle C, Liu F (2005) Human cytomegalovirus expresses novel microRNAs during productive viral infection. Cell Microbiol 7(11):1684–1695
88. Umbach J, Kramer M, Jurak I, Karnowski H, Coen D, Cullen B (2008) MicroRNAs expressed by herpes simplex virus 1 during latent infection regulate viral mRNAs. Nature 454(7205):780–783
89. Pfeffer S, Zavolan M, Grässer F, Chien M, Russo J, Ju J, John B, Enright A, Marks D, Sander C, Tuschl T (2004) Identification of virus-encoded microRNAs. Science 304(5671):734–736. https://doi.org/10.1126/science.1096781
90. Cai X, Lu S, Zhang Z, Gonzalez C, Damania B, Cullen B (2005) Kaposi's sarcoma-associated herpesvirus expresses an array of viral microRNAs in latently infected cells. Proc Natl Acad Sci USA 102(15):5570–5575
91. Pfeffer S, Sewer A, Lagos-Quintana M, Sheridan R, Sander C, Grässer F, van Dyk L, Ho C, Shuman S, Chien M, Russo J, Ju J, Randall G, Lindenbach B, Rice C, Simon V, Ho D, Zavolan M, Tuschl T (2005) Identification of microRNAs of the herpesvirus family. Nat Methods 2(4):269–276. https://doi.org/10.1038/nmeth746
92. Hussain M, Taft R, Asgari S (2008) An insect virus-encoded microRNA regulates viral replication. J Virol 82(18):9164–9170
93. Jopling C, Yi M, Lancaster A, Lemon S, Sarnow P (2005) Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. Science 309(5740):1577–1581
94. Zhou J, Yu L, Gao X, Hu J, Wang J, Dai Z, Wang J, Zhang Z, Lu S, Huang X, Wang Z, Qiu S, Wang X, Yang G, Sun H, Tang Z, Wu Y, Zhu H, Fan J (2011) Plasma microRNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma. J Clin Oncol 29(36):4781–4788
95. Xiong Y, Zhang C, Yuan J, Zhu Y, Tan Z, Kuang X, Wang X (2015) Hepatitis C virus represses the cellular antiviral response by upregulating the expression of signal transducer and activator of transcription 3 through sponging microRNA-122. Mol Med Rep 11(3):1733–1737. https://doi.org/10.3892/mmr.2014.2897
96. Cazalla D, Yario T, Steitz J, Steitz J (2010) Down-regulation of a host microRNA by a Herpesvirus saimiri noncoding RNA. Science 328(5985):1563–1566
97. Liu N, Jiao T, Huang Y, Liu W, Li Z, Ye X (2015) Hepatitis B virus regulates apoptosis and tumorigenesis through the microRNA-15a-Smad7-transforming growth factor beta pathway. J Virol 89(5):2739–2749
98. Liu N, Zhang J, Jiao T, Li Z, Peng J, Cui Z, Ye X (2013) Hepatitis B virus inhibits apoptosis of hepatoma cells by sponging the MicroRNA 15a/16 cluster. J Virol 87(24):13370–13378
99. Hon L, Zhang Z (2007) The roles of binding site arrangement and combinatorial targeting in microRNA repression of gene expression. Genome Biol 8(8):R166
100. Denzler R, Agarwal V, Stefano J, Bartel D, Stoffel M (2014) Assessing the ceRNA hypothesis with quantitative measurements of miRNA and target abundance. Mol Cell 54(5):766–776
101. Bosson A, Zamudio J, Sharp P (2014) Endogenous miRNA and target concentrations determine susceptibility to potential ceRNA competition. Mol Cell 56(3):347–359
102. Ebert M, Neilson J, Sharp P (2007) MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. Nat Methods 4(9):721–726
103. Jens M, Rajewsky N (2015) Competition between target sites of regulators shapes post-transcriptional gene regulation. Nat Rev Genet 16(2):113–126

104. Bissels U, Wild S, Tomiuk S, Holste A, Hafner M, Tuschl T, Bosio A (2009) Absolute quantification of microRNAs by using a universal reference. RNA 15(12):2375–2384
105. Calabrese J, Seila A, Yeo G, Sharp P (2007) RNA sequence analysis defines Dicer's role in mouse embryonic stem cells. Proc Natl Acad Sci USA 104(46):18097–18102
106. Mukherji S, Ebert M, Zheng G, Tsang J, Sharp P, van Oudenaarden A (2011) MicroRNAs can generate thresholds in target gene expression. Nat Genet 43(9):854–859. https://doi.org/10.1038/ng.905
107. Yuan Y, Liu B, Xie P, Zhang M, Li Y, Xie Z, Wang X (2015) Model-guided quantitative analysis of microRNA-mediated regulation on competing endogenous RNAs using a synthetic gene circuit. Proc Natl Acad Sci USA 112(10):3158–3163
108. Nielsen C, Shomron N, Sandberg R, Hornstein E, Kitzman J, Burge C (2007) Determinants of targeting by endogenous and exogenous microRNAs and siRNAs. RNA 13(11):1894–1910
109. Friedman R, Farh K, Burge C, Bartel D (2009) Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 19(1):92–105. https://doi.org/10.1101/gr.082701.108
110. Brennecke J, Stark A, Russell R, Cohen S (2005) Principles of microRNA-target recognition. PLoS Biol 3(3):e85
111. Grimson A, Farh K, Johnston W, Garrett-Engele P, Lim L, Bartel D (2007) MicroRNA targeting specificity in mammals: determinants beyond seed pairing. Mol Cell 27(1):91–105
112. Sheu-Gruttadauria J, Xiao Y, Gebert L, MacRae I (2019) Beyond the seed: structural basis for supplementary microRNA targeting by human Argonaute2. EMBO J 38(13):e101153. https://doi.org/10.15252/embj.2018101153
113. Loinger A, Shemla Y, Simon I, Margalit H, Biham O (2012) Competition between small RNAs: a quantitative view. Biophys J 102(8):1712–1721
114. Min K, Zealy R, Davila S, Fomin M, Cummings J, Makowsky D, Mcdowell C, Thigpen H, Hafner M, Kwon S, Georgescu C, Wren J, Yoon J (2018) Profiling of m6A RNA modifications identified an age-associated regulation of AGO2 mRNA stability. Aging Cell 17(3):e12753. https://doi.org/10.1111/acel.12753
115. Helwak A, Kudla G, Dudnakova T, Tollervey D (2013) Mapping the human miRNA interactome by CLASH reveals frequent noncanonical binding. Cell 153(3):654–665
116. Chi S, Hannon G, Darnell R (2012) An alternative mode of microRNA target recognition. Nat Struct Mol Biol 19(3):321–327
117. Brümmer A, Yang Y, Chan T, Xiao X (2017) Structure-mediated modulation of mRNA abundance by A-to-I editing. Nat Commun 8(1):1255. https://doi.org/10.1038/s41467-017-01459-7
118. Thomson DW, Dinger ME (2016) Endogenous microRNA sponges: evidence and controversy. Nat Rev Genet 17(5):272
119. Kartha R, Subramanian S (2014) Competing endogenous RNAs (ceRNAs): new entrants to the intricacies of gene regulation. Front Genet 5:8. https://doi.org/10.3389/fgene.2014.00008
120. Lewis B, Burge C, Bartel D (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120(1):15–20
121. John B, Enright A, Aravin A, Tuschl T, Sander C, Marks D (2004) Human MicroRNA targets. PLoS Biol 2(11):e363
122. Miranda K, Huynh T, Tay Y, Ang Y, Tam W, Thomson A, Lim B, Rigoutsos I (2006) A pattern-based method for the identification of MicroRNA binding sites and their corresponding heteroduplexes. Cell 126(6):1203–1217
123. Krek A, Grün D, Poy M, Wolf R, Rosenberg L, Epstein E, MacMenamin P, da Piedade I, Gunsalus K, Stoffel M, Rajewsky N (2005) Combinatorial microRNA target predictions. Nat Genet 37(5):495–500. https://doi.org/10.1038/ng1536
124. Kertesz M, Iovino N, Unnerstall U, Gaul U, Segal E (2007) The role of site accessibility in microRNA target recognition. Nat Genet 39(10):1278–1284
125. Mathonnet G, Fabian M, Svitkin Y, Parsyan A, Huck L, Murata T, Biffo S, Merrick W, Darzynkiewicz E, Pillai R, Filipowicz W, Duchaine T, Sonenberg N (2007) MicroRNA inhibition of translation initiation in vitro by targeting the cap-binding complex eIF4F. Science 317(5845):1764–1767. https://doi.org/10.1126/science.1146067
126. Chiu H, Martínez M, Komissarova E, Llobet-Navas D, Bansal M, Paull E, Silva J, Yang X, Sumazin P, Califano A (2018) The number of titrated microRNA species dictates ceRNA regulation. Nucleic Acids Res 46(9):4354–4369. https://doi.org/10.1093/nar/gky286
127. Poria DK, Guha A, Nandi I, Ray PS (2016) RNA-binding protein HuR sequesters microRNA-21 to prevent translation repression of proinflammatory tumor suppressor gene programmed cell death 4. Oncogene 35(13):1703–1715
128. Floor S, Doudna J (2015) Get in LINE: competition for newly minted retrotransposon proteins at the ribosome. Mol Cell 60(5):712–714
129. Chen K, Xie S, Jin W (2019) Crucial lncRNAs associated with adipocyte differentiation from human adipose-derived stem cells based on co-expression and ceRNA network analyses. PeerJ 7:e7544. https://doi.org/10.7717/peerj.7544
130. Liu Y, Wang Y, He X, Zhang S, Wang K, Wu H, Chen L (2018) LncRNA TINCR/miR-31-5p/C/EBP-α feedback loop modulates the adipogenic differentiation process in human adipose tissue-derived mesenchymal stem cells. Stem cell Res 32:35–42. https://doi.org/10.1016/j.scr.2018.08.016
131. Li R, Li B, Shen M, Cao Y, Zhang X, Li W, Tao J, Wu W, Liu H (2020) LncRNA 2310043L19Rik inhibits differentiation and promotes proliferation of myoblast by sponging miR-125a-5p. Aging 12(7):5625–5639. https://doi.org/10.18632/aging.102905
132. Li Z, Cai B, Abdalla B, Zhu X, Zheng M, Han P, Nie Q, Zhang X (2019) LncIRS1 controls muscle atrophy via sponging miR-15 family to activate IGF1-PI3K/AKT pathway. J Cachexia Sarcopenia Muscle 10(2):391–410. https://doi.org/10.1002/jcsm.12374
133. Zhang T, Liu H, Mao R, Yang H, Zhang Y, Zhang Y, Guo P, Zhan D, Xiang B, Liu Y (2020) The lncRNA RP11–142A22.4 promotes adipogenesis by sponging miR-587 to modulate Wnt5β expression. Cell Death Dis 11(6):475. https://doi.org/10.1038/s41419-020-2550-9
134. Chen Y, Li K, Zhang X, Chen J, Li M, Liu L (2020) The novel long noncoding RNA lncRNA-Adi regulates adipogenesis. Stem Cells Transl Med. https://doi.org/10.1002/sctm.19-0438
135. Sun Y, Li Y, Wang M, Yue M, Bai L, Bian J, Hao W, Sun J, Zhang S, Liu H (2020) Increased ATR expression is induced by ATR autoantibody via two axes, Klf-5/IRF-1 and circErbB4/miR-29a-5p, to promote VSMC migration. Cell Death Dis 11(6):432. https://doi.org/10.1038/s41419-020-2643-5
136. Lin J, Feng X, Zhang J (2020) Circular RNA circHIPK3 modulates the proliferation of airway smooth muscle cells by miR-326/STIM1 axis. Life Sci 255:117835. https://doi.org/10.1016/j.lfs.2020.117835
137. Shen X, Liu Z, Cao X, He H, Han S, Chen Y, Cui C, Zhao J, Li D, Wang Y, Zhu Q, Yin H (2019) Circular RNA profiling identified an abundant circular RNA circTMTC1 that inhibits chicken skeletal muscle satellite cell differentiation by sponging miR-128-3p. Int J Biol Sci 15(10):2265–2281. https://doi.org/10.7150/ijbs.36412
138. Li L, Chen Y, Nie L, Ding X, Zhang X, Zhao W, Xu X, Kyei B, Dai D, Zhan S, Guo J, Zhong T, Wang L (1862) Zhang H (2019) MyoD-induced circular RNA CDR1as promotes myogenic differentiation of skeletal muscle satellite cells. Biochim Biophys Acta 8:807–821. https://doi.org/10.1016/j.bbagrm.2019.07.001

139. Peng S, Song C, Li H, Cao X, Ma Y, Wang X, Huang Y, Lan X, Lei C, Chaogetu B, Chen H (2019) Circular RNA SNX29 sponges miR-744 to regulate proliferation and differentiation of myoblasts by activating the Wnt5a/Ca signaling pathway. Mol Ther Nucleic Acids 16:481–493. https://doi.org/10.1016/j.omtn.2019.03.009
140. Lai Y, Li J, Zhong L, He X, Si X, Sun Y, Chen Y, Zhong J, Hu Y, Li B, Liao W, Liu C, Liao Y, Xiu J, Bin J (2019) The pseudogene PTENP1 regulates smooth muscle cells as a competing endogenous RNA. Clin Sci 133(13):1439–1455. https://doi.org/10.1042/cs20190156
141. Hao S, Ma H, Niu Z, Sun S, Zou Y, Xia H (2019) hUC-MSCs secreted exosomes inhibit the glioma cell progression through PTENP1/miR-10a-5p/PTEN pathway. Eur Rev Med Pharmacol Sci 23(22):10013–10023. https://doi.org/10.26355/eurrev_201911_19568
142. Wang C, Wang L, Yang T, Su S, Hu Y, Zhong D (2020) Pseudogene PTENP1 sponges miR-214 to regulate the expression of PTEN to modulate osteoclast differentiation and attenuate osteoporosis. Cytotherapy. https://doi.org/10.1016/j.jcyt.2020.04.090
143. Ou L, Xiang T, Hao X, Wang D, Zeng Q (2020) Reduced long non-coding RNA PTENP1 contributed to proliferation and invasion via miR-19b/MTUS1 axis in patients with cervical cancer. Eur Rev Med Pharmacol Sci 24(8):4132–4144. https://doi.org/10.26355/eurrev_202004_20993
144. Sharma S, Chatterjee A, Kumar P, Lal S, Kondabagil K (2020) Upregulation of miR-101 during influenza A virus infection abrogates viral life cycle by targeting mTOR pathway. Viruses 12(4):444. https://doi.org/10.3390/v12040444