Review

The Role of N⁶-Methyladenosine Modified Circular RNA in Pathophysiological Processes

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Abstract

Circular RNA (circRNA) is a type of covalently closed and endogenous non-coding RNA (ncRNA) with tissue- and cell-specific expression patterns generated by a non-canonical splicing event. Previous reports have indicated that circRNAs exert their functions in different ways, thereby participating in various pathophysiological processes. N⁶-methyladenosine (m⁶A) methylation occurs in the N⁶-position, which is the most abundant and conserved internal transcriptional modification in eukaryotes, including mRNA and ncRNAs. Accumulating evidences confirm that m⁶A modification also exists in the circRNA and greatly affects the biological functions of circRNA. Their dysregulated expression can be a cause of various pathophysiological processes, such as spermatogenesis, myoblast differentiation, cancer, cardiovascular disease, mental illness and so on. Understanding the role of m⁶A-modified circRNAs in pathophysiological processes may contribute to better understanding the physiological mechanisms and develop new biomarkers. This review summarizes the regulatory mechanism of m⁶A modification on circRNA metabolism and the role of m⁶A-modified circRNAs in pathophysiological processes. This article may pave the way for a better understanding of the role of epigenetically modified circRNAs in pathophysiological process.

Key words: Circular RNA; N⁶-methyladenosine (m⁶A); Biogenesis; M⁶A-modified circRNA; Pathophysiological processes

Introduction

Two percent of human genome transcripts are mRNAs, the rest are noncoding RNAs (ncRNAs), which can regulate genes expression in growth and development of organisms [1,2]. Circular RNAs (circRNAs) are single-stranded and covalently closed endogenous ncRNAs that mainly exist in cytoplasm and have evolutionary conservation and specific expression pattern of tissue and developmental stage [3,4]. Furthermore, the circular structure makes circRNAs more stable and not easily degraded by ribonucleases [3].

Although circRNAs were emerged in 1976 [5,6], they were all considered as the “junk” of abnormal splicing events. Up to 2018, 30,000 circRNAs have been identified [7]. Increasing studies have shown that aberrant circRNAs are related to many human diseases, such as cardiovascular disease (CVD) [8], neurological disorders [9], diabetes mellitus [10], autoimmune disease [11] and cancers [12]. CircRNAs are promising in serving the biological targets for diagnosis and treatment due to their peculiarity. CircRNAs are involved in the regulation of biological processes by sponging disease-related microRNA (miRNA) [13] or proteins [14], or translating into proteins [15]. Currently, it has been discovered that the post-transcriptional modification N⁶-methyladenosine (m⁶A), occurring in N⁶-position of adenosine, also exists in circRNAs and participates in the metabolism and function of circRNAs [16].

Post-transcriptional modification has become a critical regulatory factor in many physiological processes and disease progression [17]. The deposition of chemically modified RNA has emerged as a basic mechanism that regulates the fate of lineages in cell transcriptome and proteome during development [18]. Around 10 million peaks collected from 672 samples have been recorded in the RNA EPItranscriptome Collection database. Even though...
the high-resolution mapping is available, only a few amounts of modifications are mapped [20]. Among those modifications, the m^6A modification of mRNA is the most prevalent regulator in the eukaryotes [21]. In addition, the dynamic and reversible m^6A modification makes it possible to regulate the complex and delicate expression of genes. Numerous studies have indicated that m^6A modification can respond to different physiological or pathological changes [18, 22].

Similar to mRNA, m^6A-modified circRNAs are also installed by a multicomponent methyltransferase complex and removed by demethylases enzymes [23]. Although relevant studies are still in infancy, growing evidences have shown that the biogenesis, decay, export and translation of circRNAs are affected by m^6A modification [24]. Although similar researches are still in a few, people have discovered the role of m^6A-modified circRNA in pathophysiological processes. For instance, a groundbreaking research has summarized circRNAs with m^6A modification are widely linked with several human diseases [23,24]. Furthermore, researches demonstrated that m^6A modification devotes to myogenesis and spermatogenesis [25,26]. This review briefly addresses current studies about circRNAs and summarizes the regulatory effect of m^6A modification on circRNA metabolism. Particularly, the role of m^6A-modified circRNAs in the formation of some pathophysiological processes is emphasized.

**CircRNA**

CircRNAs, a class of endogenous ncRNAs, are generated by variable splicing events during transcription and then form a covalently closed loop structure. Specifically, circRNAs are expressed in the different developmental stages and tissues, and play a role in plenty of important biological functions, such as affecting the development process [27], modulating immune response [28], promoting cardiac muscle repair [29], tumorigenesis or anti-tumorigenesis [30,31] and nerve injury [32]. This section will introduce the biogenesis models, types and functions of circRNAs.

**Biogenesis of circRNAs**

According to gene annotation, circRNA can be divided into three types according to gene annotation information: exonic circRNAs (ecircRNAs) [33], intronic circRNAs (ciRNAs) [34] and exon-intron circRNAs (EIciRNA) [1] (Figure 1). Although the sources of circRNAs are different, back-splicing

![Figure 1](http://www.ijbs.com)

**Figure 1. The biogenesis of circRNAs and special types of circRNA.** (A) Lariat-driven circularization. Exon skipping events result in lariat structure, then the lariat is removed, and finally circRNA is formed. Intron-pairing-driven circularization. There are complementary sequence motifs between the introns sequences flanking the exon the base complementary forms a circular structure and the introns are removed or retained to form ecircRNAs or EIciRNAs. RBP-pairing-driven circularization. The RBP interaction on the exon flanking sequence promotes the formation of circRNAs. CiRNAs. The ciRNAs were obtained from intronic lariat escape from debranching. (B) F-circRNA. CircRNA derived from the back-splicing of fusion-gene. (C) Rt-circRNA. Stop-codon read-through from read-through circRNA rt-circRNA. (D) MeccRNA. CircRNAs encoded by the mitochondrial genome.
circularization is a dominant way to produce circRNAs [35]. Generally, cis-regulatory elements and trans-acting factors regulate the biogenesis of circRNAs by controlling the splices [36].

The majorities of circRNAs are generated by protein-coding genes and contain complete exons, which implies that RNA polymerase (Pol II) participates in their transcription and spliceosome maybe involved in their biogenesis [14]. Typically, circRNAs are generated from non-canonical splicing sites, and dependent on the non-canonical splicing machinery [37]. A study in Drosophila melanogaster demonstrated that depleting one component of the spliceosome dramatically decreased the ratio of linear RNA to circRNA. The above results indicated that the processing of pre-mRNA has slowed down and nascent RNA can be used for back-splicing [14]. Additionally, Kramer et al. [38] showed that depletion of splicing factors increased the level of circRNA, and multiple factors are taken additive effects, each splicing factor is not redundant in the formation of circRNA. The back-splicing hypothesis is catalyzed by canonical spliceosome machinery [39], which means the 5' and 3' ends of transcribed exons and/or introns are covalently linked. During this process, the down-stream splice site of donor exon/intron is joined to the upstream splice site acceptor exon/intron on a pre-mRNA molecule [40] (Figure 1).

Multiple splice variants of circRNA transcripts can be generated from a single gene, depending on the exons selected during the back-splicing [41]. In the process of biogenesis, part of the RNA is transcribed from pre-mRNA, and exons are skipped as RNA folds. These structural changes generate some areas called lariat structures, in which discontinuous extrapolation zones initially become very close as the interior changes. CircRNA is formed after removing the subsequence from the lariat structure. This splicing process is called ‘lariat-driven circularization’. Due to the internalized feedback sequences of the former mRNA, the complementary pairing of introns on both sides forms circRNA. Another lariat-driven circRNA is ciRNA, whose formation processes depend on consensus RNA motifs near 5’ splice site and branchpoint [42]. Studies in Homo sapiens have identified hundreds of ciRNAs [37]. CiRNAs are largely restricted to the nucleus and regulate gene expression in a cis manner.

General speaking, endogenous human circRNAs comprise two or three exons, or even more [42], and back-splicing requires cis-elements in intron flanking circularized exons [34]. Mutagenesis results indicate that the circularization of exons is dependent on the presence of short (~30- to 40- nucleotide) inverted repeats, such as the Alu element [43]. Besides, hundreds of trans-regulators, such as RNA-binding proteins (RBPs) and miRNA-containing ribonucleoprotein complexes, control the gene expression at the posttranscriptional level in eukaryotes [44]. For example, circRNAs can be produced when the synthetic Quaking (QKI)-binding sites insert into introns [45]. RBPs in different systems and organisms can also regulate exon circularization, such as adenosine deaminases acting on RNA, nuclear factors NF90/NF110, fused in sarcoma, DExH-Box helicase 9, serine/arginine-rich proteins and epithelial splicing regulatory protein 1 [46].

**Types and characteristics of circRNAs**

Generally, circRNA can be divided into three types, and some specific sources of circRNA have recently been discovered (Figure 1). It has been demonstrated that fusion-gene is the driver in many tumors, including leukemia [47] and lung cancer [48,49]. CircRNAs derived from fusion-gene (F-circRNA) (Figure 1B), such as PML/RARα and MLL/AF9 fusion genes, have a carcinogenic effect in vivo model [45]. F- circEA-2a, derived from EML4-ALK-v3b with ‘AA’ motif at the junction site, promotes the migration and invasion of non-small cell lung cancer cell and acts as a diagnostic marker for lung cancer [48,49]. Additionally, Wu et al. [50] demonstrated that F-circSR1 and F-circSR2 originated from the same fusion gene solute carrier family 34 member 2 and reactive oxygen species proto-oncogene 1 (SLC34A2-ROS1), both of which promoted the migration of lung cancer cells, but did not affect cell proliferation.

Commonly, mRNA translation will not terminate until meet the stop codon. However, in some unconventional conditions, the ribosome goes past the stop codon and continues translating into an otherwise untranslated region (UTR) of a transcript, which is known as ‘stop-codon read-through’ [51]. It is reported that the transcriptional read-through process is related to the formation of circRNA. Genes that interfere with the regulation of transcriptional termination may contribute to the production of transcriptional read-through products and promote the generation of circRNA from downstream gene sources [52]. Moreover, based on more than 2,000 human tumor specimens from different tissue sources, and a novel type of circular RNA transcript was uncovered, termed as read-through circRNA (rt-circRNA) (Figure 1C) [12]. Lately, Liu et al. [53] reported that the mitochondrial genome of humans and mice encode hundreds of circRNAs. These meccRNA (Figure 1D) act as a molecular chaperone to facilitate nuclear-encoded proteins entry into
mitochondria. This is the first report of circRNA encoded by mammalian mitochondrial genome.

**Functions of circRNAs**

**Modulate miRNA functions**

Only small parts of the biological function of circRNAs have been investigated, one of the most typical mechanisms is to act as a miRNA sponge [13]. Many circRNAs have specific binding sites with miRNAs, which decreased miRNA activity and increase miRNA-target gene activity. Piwecka et al. [54] found that miR-7 expression was significantly decreased in CDR1as knockout mice, while some targets of miR-7 increased in CDR1as-knockout mice brain. Recently, it was demonstrated that Cyrano binds and targets miR-7 for degradation in CRISPR-Cas9 engineered mice [55]. This effect of Cyrano on miR-7 indirectly modulated the degradation of CDR1as by miR-671, which reveals a molecular regulatory network composed of non-coding RNAs.

Many studies illustrated that circRNAs participate in pathological conditions by sponging miRNAs. For example, high expression of circRNA-000284 in cervical cancer tissues promotes the migration, proliferation and invasion of cancer cells through the sponge of miR-506 [56]. CircRNAs, hsa_circ_001564 and hsa_circ_0009910 can facilitate osteosarcoma cell proliferation, migration and invasion by targeting miR-29c-3p and miR-449a, respectively [57, 58]. CircSEPT9 activates the LIF/STAT3 pathway by competitively binding to miR-637 in triple-negative breast cancer [59]. Jost et al. [60] successfully designed an artificial circRNA that can sponge miR-122 in hepatitis C virus (HCV) cells, thereby preventing the formation of HCV viral proteins and alleviating hepatitis C. In neonatal mouse ventricular cardiomyocytes, circRNA-000203 aggravated cardiac hypertrophy via specific adsorption miR-26b-5p and miR-140-3p [61]. Notably, most previously identified mammalian circRNAs are expressed at low level and do not have miRNA binding sites. Thus, circRNAs may not only act as miRNA sponges [35]. The latest findings showed that hsa_circ_008558 (circLONP2) can regulate the intercellular miRNAs maturation and metastasis, accelerating the metastasis of colorectal cancer cell to other organs [62].

**Regulate parental gene transcription**

Some scholars have pointed out that circRNAs also can participate in gene transcription regulation. ANRIL is an antisense ncRNA in cyclin-dependent kinase 4 inhibitor (INK4) locus and is found in melanoma [63]. Increased expression or mutation of ANRIL is associated with coronary atherosclerotic heart disease and atherosclerosis [64]. ANRIL also inhibits the transcription of the INK4 and its alternative reading frame (ARF) by interacting with the polycomb group complex. Burd et al. [65] speculated that the circular ANRIL (cANRIL, product of ANRIL back-splicing) may regulate the expression of INK4/ARF. Most ciRNAs are abundant in the nucleus and have few miRNA target sites. Importantly, knockdown of ciRNAs hinders the corresponding parent gene transcription. Ci-ankrd52 (ciRNA from the gene ANKRD52) deposits in the transcription initiation region of the gene, associates with the Pol II extension mechanism, and promotes the function of RNA Pol II [34].

In addition, ElciRNAs, such as ElciPAIP2 and ElciEIF3J, promote host gene transcription by interacting with the U1 small nuclear ribonucleoproteins (snRNP) in RNA-RNA conjunction to form the ElciRNAs-U1 snRNP complex [66]. In Arabidopsis, exon 6 of the SEPALLATA3 gene cyclizes and forms an R-loop structure of RNA-DNA hybrid complex by circRNA strongly binding the cognate DNA locus of host gene. R-loop structure inhibits the transcription of this region and allows the exon skipping [67]. Subsequently, this circRNA functions by modulating the alternative back-splicing of parental transcript [68]. All these functions suggest that circRNA can bind to genomic DNA to regulate alternative splicing and cis-modulate diseases genes.

**Interact with proteins**

Some circRNAs have one or more RBPs sites, which can be used as proteins sponges to isolate and inhibit the biological function of proteins. For example, cADR1as and sex-determining region Y circRNAs can bind to miRNA response factor Argonaute to be degraded [68, 69]. MBL circRNA (circMbl) flanking intron has many muscleblind (MBL) binding sites. Studies have shown that MBL is related to the biological synthesis of circMbl. When MBL protein is redundant, it will reduce the level of its own mRNA by promoting the generation of circMbl [14]. CircPABPN1 is derived from the poly(A) binding protein 1 (PABPN1) gene, and the Hu-antigen R (HuR) regulates the abundance of PABPN1 instead of circPABPN1. Interestingly, circPABPN1 and PABPN1 competes for the HuR protein binding sites [70].

Furthermore, circRNAs participate in a variety of physiological processes by interacting with proteins. Du et al [71] found circ-Foxo3 could be retained in the cytoplasm through interactions with anti-aging and anti-stress protein-related factors, such as inhibitors of DNA-binding 1 (ID-1) protein, focal
adhesion kinase and hypoxia inducible factor 1α, thereby hindering the progress of the corresponding resistance. By binding to mouse double-minute 2 (MDM2) and p53, circFoxo3 can promote the ubiquitination of p53 induced by MDM2, leading to the overall degradation of p53 [72]. Recently, some studies have found that two or more proteins are assembled into greater protein complexes, such as enzymes and their substrates, in which circRNA can serve as protein scaffolds [73]. For example, circ-Foxo3 forms a ternary complex with p21 and CDK2, which can inhibit the function of CDK2 and block cell-cycle process [74]. Another example is the acute myeloid leukemia (AML)-associated circMYBL2. This circRNA regulates the translation efficiency of oncogene FMS-like tyrosine kinase-3 (FLT3) by recruiting PTBP1, resulting in progression of FLT3-internal tandem duplication AML [75].

Designing an exogenous circRNA to target cancer, diabetes, or other disease-related protein may be useful for medical treatments.

Translate into proteins

Although circRNAs have been well-described as miRNA sponges, many of them do not contain miRNA capture sites. Researches indicated that many circRNAs are generated from exons localized in the cytoplasm, suggesting that they can be translated into peptides by loading into ribosomes [33]. The genome of hepatitis delta (D) virus (HDV) has been identified with a natural translatable circRNA. The core circRNA of HDV encoded the HDV antigen, which plays critical role in the hepatitis disease development [76]. Guo et al. [77] found that circRNAs could be translated in human osteosarcoma U2OS cells, while most circular isoforms are far less efficiently compared to their linear isoforms. Because circRNAs do not have a 5’ cap structure and cannot be translated into protein through cap-dependent mechanisms.

Another way of RNA translation is to use the internal ribosome entry site (IRES) sequence as an internal ribosome entry sites for translatable circRNAs [78]. Artificial circRNAs with a RES can be translated in vitro or in vivo [79-81]. The covalently closed circular RNA is found in rice yellow mottle virus, which possesses an internal ribosome entry site and utilizes two (or three) open reading frames (ORFs) that directly translate into a 16-kDa highly basic protein [82].

Although many circRNAs have ORFs and with an upstream IRES elements, 46 of these are translated into corresponding proteins according to mass spectrometry [83]. So far, only circular RNA zinc finger protein 609 (circ-ZNF609), circMb1, circ-FBXW7, circPINTTaxon2, circ-SHPRH and circβ-catenin have the coding ability in related studies. Circ-ANF609 is related with heavy polysomes and can be translated in an IRES-dependent mode, even though the translation activity of the circular template is much lower than the linear counterpart template [84]. Furthermore, decreases of circ-ZNF609 expression cause damage to the proliferation of human and mouse myoblasts, and the translation of circ-ZNF609 can be significantly induced by heat shock. Circ-Mb1 shares the same start codon with the host RNA, encoding a peptide in a cap-independent manner in Drosophila, starvation can regulate the circ-Mb1 production and/or stability depending on 4E-BP and FOXO proteins [15].

Additionally, circ-FBXW7 [85], circ-SHPRH [86], 87] and circPINTTaxon2 [88] can produce FBXW7-185aa, PINT87aa and SHPRH-146aaa, respectively. All of them can inhibit human glioblastomas. Circ-FBXW7 is extremely abundant in the brain, and IRES drives a 21-kDa protein named FBXW7-185aa. FBXW7-185aaa directly interacts with de-ubiquitinating enzyme USP28, which can stabilize the oncoprotein c-Myc by inhibiting FBXW7α (the most abundant isoform of the FBXW7 gene) activity. FBXW7-185aaa can inhibit proliferation and cell cycle of U251 and U373 cell lines by this way [85]. Two reports have successively reported that circ-SHPRH (originate from the sucrose non fermenting 2 histone linker PHD RING helicase (SHPRH) gene) employs overlapping genetic codes to encode a 17-kDa peptide named SHPRH-146aa [85, 86]. Theoretically, SHPRH-146aaa protects full-length SHPRH from degradation by ubiquitin proteasomes. E3 ubiquitin protein ligase SHPRH increases its anti-tumor function by targeting proliferating cell nuclear antigen. Finally, circPINTTaxon2 is generated from long intergenic non-protein-coding RNA P53-induced transcript gene and encodes a peptide named PINT87aa, which can inhibit multiple oncogenes though interacting with polymerase associated factor complexes and act as a tumor suppressor [88].

Polymerase associated factor complex is involved in the recruitment of RNA Pol II and the transcriptional elongation of downstream genes [89]. Circβ-catenin encodes a 370-amino acid β-catenin isoform by IERS, which prevents β-catenin from phosphorylation and degradation by antagonizing glycogen synthase kinase 3β and subsequently promotes tumor growth through activating the Wnt pathway [90]. The information about circRNA-encoded proteins is scattered in many published papers, which is not conducive to further exploration on circRNA translation. Liu et al. [91] presented an ncEP database, which recorded all published articles containing proteins or peptides encoded by ncRNAs. These
discoveries imply that the coding potential of circRNAs has been largely disregarded, which may help to deepen our understanding of circRNAs.

**Pseudogenes derived from circRNAs**

Around 19,000 conserved pseudogenes have been revealed in human by sequencing efforts and their transcription patterns display tissue and pathological condition specificity [92]. Researchers developed computational pipeline to identify circRNAs-originated pseudogene, called CIRCpseudo, to determine whether stable circRNAs can be retrotranscribed and integrated into the host genome. Dozens of circRNAs-derived pseudogenes have been identified [93]. Among them, at least 33 circRNAs at the ring finger and WD repeat domain 2 (RFWD2) locus-derived pseudogenes (circRFWD2) were found in different mouse strains, characterized by the exon 6-exon 2 anchor in a reversed order. The pseudogene derived from circSATB1 can specifically bind to CCCTC-binding factor and/or Rad21-binding sites in several mouse cell lines to regulate gene expression. In addition, the insertion of retrotransposed circRNAs into the genome changes the composition of genomic DNA and regulates the potential for gene expression [93].

**CircRNA acts as a ribozyme**

Satellite virus, viroid and HDV RNA exist in a circular form, and RNA is part of a replication ring that contains self-dividing sequences (ribozymes). Through the research of the classical type I hammerhead ribozyme (HHR) motif in diverse metazoans genomes, a novel and conserved small ribozyme has been found to efficiently synthesize circular RNA [94]. A systematic analysis of the genomes of cnidaria (a coral), mollusca (a mussel) and chordata (a salamander) has revealed that there are abundant type I HHR motifs in the DNA tandem repeats of their genomes, with a length of about 170-400 nt, mostly in the form of linear and circRNAs. These modifications confirmed the existence of novel natural pathways for circRNA biosynthesis through a conserved autocatalytic RNA in metazoan.

**M6A modification**

Epigenetic modifications are involved in cell fate and respond to environmental stimuli by regulating gene expression [95]. These modifications are implicated in the development of organism [18,96,97]. While the role of epitranscriptomic modifications in gene regulation is scarcely acquainted. Recently, considerable studies have revealed the role of post-transcriptional RNA modifications in modeling an epitranscriptomic landscape of gene expression [23,98]. RNA modifications tend to deposit in highly abundant RNA species to regulate RNA splicing, transport, translation and turnover [99].

There are more than 170 known RNA modifications, such as m6A, N7-methyladenosine, inosine, 5-methylcytidine, 5-hydroxymethylcytidine, pseudouridine, N6,2'-O-dimethyladenosine, N4-acetylcytidine, N5-methylguanosine, 8-oxoguanosine and 2'-O-methyl [100]. Among them, m6A is the most abundant modifications of mRNAs found in all eukaryotes [22]. Since around 20-40% of mammalian transcripts are m6A methylated, and the methylated mRNAs tend to have multiple m6A modifications [21]. Furthermore, recovering the mechanisms of deposition, removing and recognition RNA modifications (more preferably known as writers, erasers and readers, respectively) have helped to understand the fates of those post-transcriptional modifications in cellular processes, such as cell growth, body development and diseases.

**Regulators of m6A**

M6A is similar to DNA and histone methylation, a dynamic and reversible event [101], and the methyltransferases and demethylases coordinate to regulate the deposition and decay of m6A, also named with m6A writer and m6A eraser respectively (Figure 2). M6A is installed by a multiprotein methyltransferase complex (MTC, also termed m6A ‘writer’). The core of MTC is a heterodimer core catalytic subunit composed of methyltransferase-like 3 (METTL3) and methyltransferase-like 14 (METTL14) [102-104]. There are many other accessory subunits, including wilms tumor 1 associated protein (WTAP) [105], vir-like m6A methyltransferase-associated (also known as Virilizer or KIAA1429) [106], RNA binding motif protein 15 [107], cbl proto oncogene-like protein 1 (also known as Hakai) [108] and zinc finger CCCH domain-containing protein 13 (ZC3H13) [107]. METTL3 is the catalytic subunit and METTL14 acts as the RNA-binding platform. Moreover, the METTL3 homolog methyltransferase-like 16 (METTL16) can catalyze the m6A onto the U6 small nuclear RNA (snRNA) and some structured RNAs [110]. In addition to the above cofactors, other catalytic subunits join the methyltransferase complex, which is designed for precise post-transcriptional regulation [85]. Two demethylases, fat mass and obesity-associated protein (FTO) and α-ketoglutarate-dependent dioxygenase alkB homolog 5 (ALKBH5), can remove the m6A modification, acting as erasers [101,111].
Figure 2. Mechanism of m6A. The dynamic modification of m6A modification is installed by the methyltransferase complex (writers) composed of METTL3, METTL14, WTAP, KIA1429, ZC3H13 and RBM15/RBM15b, and removed by RNA demethylases (erasers) FTO and ALKBH5. Methyl-specific binding proteins (readers) in are the YTH family, which can achieve the biological functions of m6A modification.

The m6A modification realizes its biological function specific recognition and binding of RNA binding proteins (readers), thereby affecting RNA fate by regulating RNA metabolism. Several proteins or complexes that specifically recognize m6A sites have been identified, including YT521-B homology (YTH) domain-containing protein [112], eukaryotic initiation factor 3 [96], IGF2 mRNA binding proteins (IGF2BP) families [113] and heterogeneous nuclear ribonucleoprotein protein family. The YTH domain can recognize m6A through a conserved aromatic cage and another two proteins FMRA, LRPPRC ‘read’ this modification [114].

Characteristics and functions of m6A

M6A modification exists in different types of mammalian cell, including blood, liver, neuronal and muscle cells and so on. The deposition of m6A modification affects the process of cell metabolism, transcription, splicing, translation, degradation and localization.

The distribution of m6A modification on mRNA is sequence-specific and often appears on a consensus RNA motif of RRACH (R = G or A; H = A, C or U), coding sequence, 3'UTRs, especially near stop codons [115]. M6A modification can cause changes in RNA secondary structure by affecting RNA pairing, regulate RNA stability and its interaction with proteins [116,117]. M6A is also highly enriched in the exon region near the splicing site, and has significant spatial overlap with the splicing factor SRSF1 and SRSF2 RNA binding sites. Experimental studies have revealed that m6A-modified exons tended to be retained during splicing [118]. Ke et al. [119] illustrated the molecular mechanism of m6A-modified exons being retained during the splicing process: m6A binding protein YTHDC1 promotes its retention by promoting SRSF3 and suppressing SRSF10 in combination with m6A-modified exons. M6A also shows a tendency of enrichment in the last exon of a transcript, which may be related to the regulation of the alternative polyadenylation of mRNA. The unstable pairing of m6A-U causes the melting of double-stranded RNAs and transformation of secondary structure.

M6A methylation on circRNAs metabolism

Chemical covalent modification of nucleotides molecules is a ubiquitous life process and many studies have illustrated chemical modifications in DNA and RNA. There have been identified more than 100 types of chemical modifications of mRNAs, rRNAs, snRNAs and snoRNAs have been identified in organisms. It has been reported that linear mRNA
and lncRNA have m^6A modification. Currently, m^6A modification has been identified and characterized in circRNA, but the potential regulatory mechanism has not been fully elucidated [120]. The researches on circRNAs with m^6A-modification, and the function of these m^6A-modifications are described in Figure 3.

M^6A on circRNA translation

Studies have shown that circRNAs have coding potential. Yang et al. [16] described a new mechanism that circRNA with m^6A residues can be translated in a cap-independent manner (Figure 3A). These translatable circRNAs contain a large number of m^6A consensus motifs (13% of total circRNAs), and a single m^6A site is enough to initiate circRNA translation. The translation of m^6A-modified circRNA depends on IF eIF4G2 (non-canonical eIF4G) protein and m^6A reader YTHDF3. The m^6A-driven translation is reversible, because methyltransferase METTL3/14 can increase the translation efficiency, while inhibited by the demethylase FTO. Moreover, mRNA with m^6A modification in its 5’UTR can be translated under specific cellular stress (such as amino acid starvation) and through 5’cap-independent way to regulate the translation [121]. Here, they proved that m^6A can mediate the generation of circRNA and reports a new mechanism that relies on circRNA to achieve the stable expression of protein products after linear RNA deletion. Di Timoteo et al. [121] further demonstrated this point by taking circ-ZNF609 as a study case. The generation of circ-ZNF609 modified by m^6A is regulated by METTL3/YTHDC1, and the recognition of m^6A site by YTHDF3 and eIF4G2 promotes circ-ZNF609 translation.

![Figure 3. M^6A modification affects the function of circRNAs.](http://www.ijbs.com)
Endoribonucleolytic cleavage of circRNA

m^6A is involved in regulating multiple steps of mRNA modification, including stability, and the degradation of mRNA containing m^6A modification is mediated by YTHDF2 (Figure 3B). In detail, it depends on the presence of the adaptor protein heat-responsive protein 12 (HRSP12)-binding site in the messenger ribonucleoprotein. If it exists, deadenylation pathway mediated by CCR4/NOT complex is activated, leading to deadenylation and decay of m^6A-containing mRNAs [122] or endoribonucleolytic cleavage pathway by the YTHDF2-HRSP12-RNase P/mitochondrial RNA-processing (MRP) (endoribonuclease) complex causing degradation of YTHDF2-bound RNAs [123]. Comparing to mRNA, circRNAs have a covalently closed loop and do not have a 3’ polyadenylated tail, they are naturally more stable than their homologous linear RNAs in both intracellular and extracellular environments and their degradation can be avoided [124]. Therefore, circRNAs can only be degraded by endoribonucleolytic cleavage. Park et al. [123] demonstrated that circRNAs containing m^6A can be decayed through YTHDF2-HRSP12-RNase P/MRP-mediated endoribonucleolytic cleavage. The abundance of circRNAs containing m^6A increased after a component of RNase P/MRP was down-regulated [123].

Promote the nuclear export of circRNA

Following biogenesis, most circRNAs are efficiently exported to cytoplasm. However, the intron-containing circRNAs accumulate in the nucleus [68,69]. Some nondividing cells (e.g. neurons) highly express circRNAs. Therefore, the transport process regulates the localization of circRNAs, depending on nuclear envelope breakdown during mitosis. Based on RNA interference screening in Drosophila, the interference of Hel25E significantly led to the enrichment of circRNA in the nucleus. Further identification showed that Hel25E was an important regulator of post-transcriptional nuclear export of circRNAs. In human cells, circRNAs are transported from the nucleus in a transcript-length-dependent manner to the cytoplasm via Hel25E homogenous proteins: ATP-dependent RNA helicase DDX39A (also known as nuclear RNA helicase URH49 or URH49) and spliceosome RNA helicase DDX39B (also known as DEAD box protein UAP56 or UAP56) [125].

Knockout of m^6A demethylase ALKBH5 accelerates the nuclear export of mRNA (Figure 3C) [126]. Mechanism studies have proved that YTHDC1 interacts with splice factor SRSF3 to recruit NXF1, thereby promoting the nucleation of m^6A mRNA [127]. Chen et al. [128] found that m^6A-modified circRNA NOP2/Sun RNA methyltransferase family member 2 (NSUN2) locus (circNSUN2) exports from the nucleus to cytoplasm by binding to YTHDC1. Then cytoplasmic circNSUN2 interacts with RBP and insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2) to promote the stability of high mobility group AT-hook 2 (HMGA2) transcript [128]. Compared with paracancerous tissues, circNSUN2 is highly expressed in colorectal cancer tissues during the progression of lymph node metastasis and liver metastasis in colorectal cancer, and the expression level of circNSUN2 gradually increases. Further, in vivo nude mouse metastasis and in vitro cell function experiments show that circNSUN2 promotes the metastasis of colorectal cancer tumours. This circRNA binds to the m^6A binding protein YTHDC1 in the nucleus, and YTHDF1 regulates the nuclear localization of circNSUN2 in an m^6A-dependent manner. The cytoplasm of circNSUN2 can bind to the RNA-binding protein IGF2BP2 and the downstream HMGA2 (a member of the high motility group, HMG) mRNA to form a circNSUN2/IGF2BP2/HMGA2 RNA-protein ternary complex, which promotes the stability of HMGA2 mRNA. Ultimately, m^6A modified circNSUN2 promotes liver metastasis of colorectal cancer tumors by accommodating the stability of HMGA2 mRNA [128]. It is suggested that the interacting proteins can help to discover inner mechanism of circRNAs.

m^6A-modified circRNA associated with pathophysiological processes

With the gradual understanding of the structure and function of m^6A modification and circRNA, researchers have investigated the role of m^6A-modified circRNAs in physiological and pathological processes. Additionally, many evidences were showed that m^6A-modified circRNA are related with the progress of various cancer [24, 128-132], immune response [133], CVD [134], mental illness [135], myoblast differentiation [25], spermatogenesis [26] and so on. The pathophysiological processes regulated by m^6A-modified circRNAs are summarized in here.

m^6A-modified circRNA in pathological processes

Although most m^6A-modified circRNAs are thought play a role in pathological processes, the function of m^6A modification on circRNA is unknown. Table 1 lists the proposed mechanism and functions of m^6A-modified circRNAs, and is discussed below.
and may be a potential target for the HCC therapy RNA, which induces sorafenib resistance by [130].

of HCC through the m 6A-YTHDF3-Zeb1 mechanism, (originate from KIAA1429) can accelerate the progress rate worldwide [137]. In HCC, circ_KIAA1429 modified circRNA plays a role in GC progression circPVRL3, indicating that circPVRL3 has coding function in different diseases, and many of them highlighted a role in various cancer [17,136]. Cancer is a leading cause of mortality worldwide [137]. Based on the structure and function of m6A-modified circRNA, researchers have found that they play an important role in the progression, proliferation and metastasis of cancers, such as colorectal cancer [128], gastric cancer [129], hepatocellular carcinoma (HCC) [130,131] and poorly differentiated gastric adenocarcinoma [132].

A study on gastric cancer (GC) found that knockdown of circRNA poliovirus receptor-related 3 (circPVRL3) significantly promoted cell proliferation [129]. The internal ribosomal entry sites, open reading frame and m6A modification are present on circPVRL3, indicating that circPVRL3 has coding potential. Overall, this study pointed to the m6A-modified circPVRL in the carcinogenesis [129]. 30 upregulated circRNAs and 35 downregulated circRNAs were evaluated in poorly differentiated gastric adenocarcinoma (PDGA) by circRNA microarray. The level of m6A was positive relative with circRNA in PDGA, which indicated that m6A-modified circRNA plays a role in GC progression [130].

Liver cancer has a high morbidity and mortality rate worldwide [137]. In HCC, circ_KIAA1429 (originate from KIAA1429) can accelerate the progress of HCC through the m6A-YTHDF3-Zeb1 mechanism, and may be a potential target for the HCC therapy [131]. In addition to modifying m6A modification process, circRNA can also affect the stability of circRNA via changing the methylation states. In HCC, circRNA-SORE acts as a competitive endogenous RNA, which induces sorafenib resistance by competitively activating the Wnt/β-catenin pathway by adsorbing miR-103a-2-5p and miR-660-3p. They found that the increasing m6A level of circRNA-SORE can maintain its stability and promotes the development of drug resistance [132]. This paper expounds the new mechanism of drug resistance in targeted therapy of liver cancer and provides new clues for finding new therapeutic targets for advanced liver cancer patients.

Cancers

Wealth of recent studies have revealed that circRNA and m6A modification exert a regulatory function in different diseases, and many of them highlighted a role in various cancer [17,136]. Cancer is a leading cause of mortality worldwide [137]. Based on the structure and function of m6A-modified circRNA, researchers have found that they play an important role in the progression, proliferation and metastasis of cancers, such as colorectal cancer [128], gastric cancer [129], hepatocellular carcinoma (HCC) [130,131] and poorly differentiated gastric adenocarcinoma [132].

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Lung cancer is the leading cause of cancer death in men [138]. Recently, a study indicated that circNDUFB2, derived from NADH: ubiquinone oxidoreductase subunit B2, is frequently downregulated in non-small cell lung cancer (NSCLC), and negatively correlated with the malignant characteristics of NSCLC. CircNDUFB2 acts as a scaffold of tripartite motif containing 25 and IGF2BPs to form a ternary complex, which promotes the degradation of IGF2BPs and inhibits the growth and metastasis of NSCLC cells. Furthermore, m6A-modified circNDUFB2 can enhance this effect [139]. In addition, circNDUFB2 recognized by RIG-I can active RIG-I-MAVS signaling cascades, thereby recruiting immune cells into the tumor microenvironment. Apart from enhancing IGF2BPs stability, the down-regulated circNDUFB2 in NSCLC also leads to immune evasion and promotes tumor progression [139].

In addition, some m6A-modified circRNAs have not yet clarified their functions. In esophageal squamous cell carcinoma (ESCC), circ-SLC7A5 was significantly up-regulated in the plasma of ESCC patients and high levels of circ-SLC7A5 are correlated with shorter survival time. Bioinformatics predicts that circ-SLC7A5 has m6A modification structure with translation potential [140]. Zhou et al. [32] developed a computational pipeline together with experimental results, which revealed m6A circRNAs are widely spread in circRNAs (they accounts for 41% of the total.
circRNAs) and are significantly different between human embryonic stem cells (hESCs) and HeLa cells. Moreover, even if circRNAs are originated from the same mRNA, they cannot be detected in different cell types. Up to 65% of m6A-modified circRNA derived from HeLa cells cannot be detected in hESCs. Furthermore, about 41% of HeLa-generated circRNA do not appear in hESCs. Zhou et al. [32] also found some other features of m6A-modified circRNA: 1) the distribution of m6A modification sites on circRNAs and corresponding mRNAs are different; 2) the parental exons of m6A circRNAs are relatively greater than those of non-methylated circRNAs; 3) circRNAs share the same m6A modification enzymes with linear RNA; 4) the host mRNA is unstable. The m6A expression level is negatively associated with circRNA expression. This article found that circRNA has a cell-specific m6A modification state, which can be used to diagnose diseases.

Immune response

CircRNA is automatically spliced and cyclized after in vitro transcription to transfected cells. Unexpectedly, this type of circRNA can induce the activation of innate immune response and inhibition of the RNA virus infection process by retinoic acid-inducible gene-I (RIG-I) [134]. Endogenous circRNA with m6A modification can suppress innate immune responses by inhibiting RIG-I activation [141]. When cells are stimulated by polyinosinic acid-polycytidylic acid or infected by viruses, the endonuclease RNase L activates and degrades circRNA molecules. Protein kinase (PKR) is released and activates the downstream antiviral mechanism systemic lupus erythematosus (SLE). The expression of circRNA in the peripheral blood mononuclear cells (PBMC) of SLE patients decreased and the activity of PKR increased. However, overexpression of circRNA in PBMC derived from SLE patients can reduce the activity of PKR, which may be helpful for the treatment of autoimmune diseases such as SLE [134].

Chen et al. [134] found that the lack of sample purity might be caused by the non-specificity of the substituted linear RNA with 5’ phosphate effect. Thus, Chen et al. [134] did some other experiments to show that their research system is reliable and produced a novel mechanism. The m6A reader YTHDF2 may be reason for the RNA degradation [19, 24]. Studies have shown that m6A modification controls immune responses [142]. CircE7 was modified by m6A and translated to produce E7 oncoprotein [143]. CircE7 exists in TCGA RNA-Seq data of human papillomavirus (HPV)-positive cancer cells and in cell lines where only free HPV is present. These results prove that virus-derived that can encode proteins has biological functions and is related to certain HPV transformation characteristics [144].

Wesselhoeft et al. [144] found that exogenously synthesized circRNA transfected into cells did not induce toll-like receptor/RIG-I mediated innate immune response, and circRNAs can more effectively translate into proteins in mouse tissues. Deposition of m6A modification in circRNA can recruit and bind YTHDF2, inhibit the activation of RIG-I/K63-Ubn/circRNA complex and do not induce the innate immune response [144]. Collectively, these finding proved that m6A modification of circRNAs is a key regulator in circRNA related immune responses.

CVD

CVD causes 17.5 million annual deaths and increases the burden of public health [145]. Cell death is one of the critical pathological mechanism of atherosclerosis (AS). IFN regulatory factor (IRF)-1 has been demonstrated play a critical role in regulating cell death of AS. The relative RNA expression level of hsa_circ_0029589 in macrophages of acute coronary syndrome decreased, while the m6A level of hsa_circ_0029589 and the m6A METL3 were significantly increased [146]. In addition, the overexpression of IRF-1 inhibited the expression of hsa_circ_0029589 in macrophages, and simultaneously induced the expression of m6A and METL3. Overexpression of hsa_circ_0029589 or inhibition of METTL3 can significantly increase the expression of hsa_circ_0029589 and reduce macrophage apoptosis [146]. Increased m6A abundance in hypoxia mediated pulmonary hypertension (HPH) reduces the total circRNAs abundance in hypoxia in vitro [147]. M6A affects the co-expression network of circRNA-siRNA-mRNA during hypoxia. Specifically, the m6A-modified circXpo6 and circTmtc3 may be used as HPH biomarkers due to their different enrichments in specific pathological condition [146]. The difference of m6A circRNAs in cells or tissues suggests that they may be involved in progression of CVD or act as a pathological marker.

Age-related cataract

Age-related cataract (ARC) is the leading cause of world blindness, which causes ~50% blindness worldwide [148]. Few circRNA has been identified in cataract. For instance, circKMT2E sponges miR-204 to regulate the pathogenesis of diabetic cataract [149]. Total circRNA expression level was decreased in cortical of ARC (ARCC). Compared with non-m6A modified circRNAs, the expression of highly abundant m6A circRNAs were mostly reduced. Bioinformatics analysis predicted that ALKBH5 was significantly upregulated in lens epithelium cells.

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(LEC) of ARCC among the five major methyltransferases. Those results indicate that M6A modification of circRNAs may be associated with LEC lesions by regulating genes/pathways related to the onset of ARC [150].

Major depressive disorder

Major depressive disorder (MDD) is a severe mental disorder with high incidence [151]. Currently, several studies have demonstrated circRNAs play a role in MDD. CircDYM is originated from DYM gene exon 4, 5, 6 circulation, and was found to be significantly decreased in plasma of MDD patients [152]. Huang et al. found that circRNA stromal antigen 1 and 2 (STAG1) play a crucial role in attenuating depressive-like behaviors. circSTAG1 can bind ALKBH5 to inhibit its nuclear entry, thereby changing the m6A modification of total RNA and increasing the level of m6A modification of RNA, including fatty acid amid hydrolase (FAAH) mRNA [135]. The expression of circSTAG1 in the hippocampus of CUS depressed mice was was significantly reduced, and the m6A modification of FAAH mRNA was changed by ALKBH5, which ultimately led to the depression phenotype. 345-395aa of ALKBH5 may be the region that binds to circSTAG1. This study revealed that circRNA regulates the process of m6A modification by combining to m6A modification enzyme.

m6A-modified circRNA in physical processes

Myogenesis

Increasing evidences have shown that circRNAs are abundant in skeletal muscles during the differentiation myoblasts, and the global expression level of circRNA changes dynamically [153]. Additionally, several circRNAs (such as circ-ZNF609 and circular RNA supervillin have been proved played an important role in the differentiation and development of skeletal muscle [154]. Here, newly study has found that 581 circRNAs are differentially expressed between skeletal muscle C2C12 myoblasts and myotubes. These myogenic-specific genes, 91 miRNA and the top 30 upregulated circRNAs forming a regulating network with 239 edges. Among the 581 circRNAs, 224 circRNAs have been identified as having coding potential. In addition, the number of m6A motifs in 224 circRNAs with encoding potential was also determined. Totally, 44 circRNAs had an m6A motif, 43 circRNAs had two m6A motifs, and 137 circRNAs had three or more m6A motifs. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analyses were performed that 75 circRNAs based on the linear counterparts were related with actin cytoskeleton and metabolic pathways [25]. These annotations may offer novel insights in the development and differentiation of skeletal muscle and provide new therapeutic strategies foe muscular diseases.

Spermatogenesis

Spermatogenesis is a highly complex and specialized cell differential process during which diploid spermagional stem cells produce spermatozoa [155]. Studies have shown that depletion of m6A writers (Mettl3 and Mettl14 or their homologs) can impair gametogenesis in multiple organisms [156]. Recent research demonstrated that m6A is dynamically regulated and plays an important role in shaping gene expression during spermatogenesis [157]. Moreover, a large number of circRNA will be generated from the thick line stage after meiosis to the round cell stage and the corresponding linear mRNA expression decreases during spermatogenesis [26]. Tang et al. [26] studied the developmental process of male germ cells in mice that m6A modification can promote the formation of ORF-carrying circRNA. Sequencing analysis during spermatogenesis has found that a large number of circRNAs were generated while the corresponding linear mRNA was decreased. Some circRNAs often have high levels of m6A modification on both sides of the reverse junction point, and m6A is often enriched around the ORF start and stop codons of the mRNA. Interestingly, Tang et al. [26] found that nearly half of these circRNAs carry longer ORFs whose start codon is modified by m6A to bind ribosomes. Hundreds of peptides encoded by these circRNAs were detected by liquid chromatography and mass spectrometry. This research not only proved that m6A can mediate the generation of circRNA, but also discovered a novel mechanism for stable expression of circRNA-dependent protein product after linear RNA deletion.

Conclusions and future directions

Recent researches on circRNA with m6A modification uncovered that epigenetic modification can also affect the circRNAs involved cellular process. CircRNA interacts with m6A-related proteins can regulate the progression and development of cancer. There are very few studies about circRNAs with m6A modification, so more understanding about the regulation and functions of this modification in circRNAs is still needed. Furthermore, m6A is most common in linear RNA and circRNA is considered to be the shaper of the RNA world [158]. These findings indicate that the m6A modification may be a means for circRNA to achieve this reshape function. Therefore, m6A modifications in circRNA can uncover more information about the function of epigenetic
modification in RNA world. However, limitations still exist in current researches. Further exploration can be conducted from the following aspects: 1) Determining the molecular mechanism of circRNA m6A modification (Are the ‘writers’, ‘erasers’ and ‘readers’ completely consistent with linear RNA?); 2) Elucidating the formation of cell types or tissues specific circRNAs (Why do circRNAs have specific modifications? Do they have a special function? Are there circRNAs that are not modified by m6A?); 3) Differentiating between specific exons and classical exons with m6A modification. Further studies are needed to unravel those mysteries.

Furthermore, this review describes the role, mechanism and application of m6A-modified circRNA in the pathophysiological processes. The most important role of circRNAs is in the development of various diseases and life processes. Nevertheless, the exact mechanism for m6A-modified circRNA in life progression is still unclear, because m6A modification can act as a double-edged sword. In spite of epigenetically modification play a significant regulatory role in the biological function of cell, while the terminal aim of medical researches is to be applied in the clinic. Designing targeted therapeutic drugs based on these newly elucidated molecular mechanisms the subject.

Abbreviations

CircRNAs: Circular RNAs; ncRNA: Noncoding RNA; m6A: N6-methyladenosine; miRNA: MicroRNA; EcircRNAs: Exonic circRNAs; CiRNAs: Intronic circRNAs; ElcircRNA: Exon-intron circRNAs; Pol II: RNA polymerase II; RBPs: RNA-binding proteins; QKI: Quaking; F-circRNA: CircRNAs derived from fusion-gene; ROS: Reactive oxygen species; SLC34A2-ROS1: Solute carrier family 34 member 2 and ROS proto-oncogene 1; UTR: Untranslated region; rt-circRNA: Read-through circRNA; HCV: Hepatitis C virus; NMVCs: Neonatal mouse ventricular cardiomyocytes; CRC: Colorectal cancer; ANRIL: The antisense ncRNA in cyclin-dependent kinase 4 inhibitor locus; INK4: Cyclin-dependent kinase 4 inhibitor; ARF: Alternative reading frame; SnRNPs: Small nuclear ribonucleoproteins; MBL: Muscleblind; circMBL: Muscleblind circRNA; PABPN1: Poly (A) binding protein 1; HuR: Hu-antigen R; MD2M: Mouse double-minute 2; AML: Acute myeloid leukemia; FLT3: FMS-like tyrosine kinase-3; 3D: Hepatitis delta (D) virus; IRESs: Internal ribosome entry sites; ORFs: Open reading frames; SHPRH: Sucrose non-fermenting 2 (SNF2) histone linker PHD RING helicase; LINC-PINT: Long intergenic non-protein-coding RNA p53-induced transcript; RFWD2: Ring finger and WD repeat domain 2; CTCF: CCCTC-binding factor; HHR: Hammerhead ribozyme; MTC: Methyltransferase complex; METTL1: Methyltransferase-like; WTAP: Wilms Tumor 1 associated protein; ZC3H13: Zinc finger CCCH domain-containing protein 13; SnRNA: Small nuclear RNA; FTO: Obesity-associated protein; ALKBH5: a-ketoglutarate-dependent dioxygenase alkB homolog 5; YTH: YT521-B homology; IGF2BP: IGF2 mRNA binding proteins; mRNP: Messenger ribonucleoprotein; MRP: Mitochondrial RNA-processing; NSUN2: NOP2/Sun RNA methyltransferase family member 2; IGF2BP2: Insulin-like growth factor 2 mRNA-binding protein 2; HMG2A: High mobility group AT-hook 2; GC: Gastric cancer; PDGA: Poorly differentiated gastric adenocarcinoma; CC: Colorectal cancer; HCC: Hepatocellular carcinoma; circPVR1L3: circRNA poliovirus receptor-related 3; ESC: esophageal squamous cell carcinoma; hESCs: Human embryonic stem cells; RIG-I: Retinoic acid-inducible gene-I; PKR: Protein kinase; SLE: Systemic lupus erythematosus; PBMC: Peripheral blood mononuclear cells; HPV: Human papillomavirus; AS: Atherosclerosis; HPH: Hypoxia mediated pulmonary hypertension; ARC: Age-related cataract; ARCC: Cortical of ARC; LECs: Lens epithelium cells; MDD: Major depressive disorder; STAG: Stromal antigen 1 and 2; FAAH: fatty acid amide hydrolase; RNase: Ribonuclease; LM: Liver metastasis; RNPs: Ribonucleoprotein particles; NSCLC: Non-small cell lung cancer.

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Competing Interests

The authors have declared that no competing interest exists.

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