Molecular Therapy
Nucleic Acids
Review

Novel insights into the interaction between N6-methyladenosine modification and circular RNA

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As the most prevalent type of RNA modification in eukaryotes, N6-methyladenosine (m^6A) can modulate RNA fates such as processing, splicing, maturation, export, stability, translation, and degradation. Circular RNAs (circRNAs), a novel type of non-coding RNA (ncRNAs) characterized by a covalently closed loop structure, play an essential role in various physiological and pathological processes. Extensive studies have revealed that m^6A modification is widespread in circRNAs and influences their biogenesis and functions. Intriguingly, circRNAs can affect m^6A modification by regulating m^6A regulatory proteins. In this review, we summarize the characteristics and biological functions of m^6A and circRNAs and focus on recent advances in the interaction of m^6A modification and circRNAs. In addition, the potential clinical applications of m^6A modification and circRNAs in diagnosis and therapeutic targets are discussed.

INTRODUCTION

Due to far-reaching advances in science and technology, many chemical modifications of DNA, RNA, and proteins have been identified, and chemical modification of RNA is the most abundant.[1] As a result of the widespread presence of >100 RNA modifications in different RNA types, including eukaryotic messenger RNAs (mRNAs), non-coding RNAs (ncRNAs), and viral RNA genomes, a new field of epitranscriptomics has arisen.[2,3] RNA methylation is one of the predominant forms of epigenetic modification, accounting for >60% of RNA modifications.[4] The common sites of RNA methylation mainly include N^6-methyladenosine (m^6A), 5-methylcytosine (m^5C), N^1-methyladenosine (m^1A) and 7-methylguanosine (m^7G). As the most prevalent type among these methylations, m^6A, which describes a methylation at the N^6 position of adenosine and enriched in the consensus sequence RRACH (R: A or G; A: m^6A; and H: A, C, or U), has become a hotspot in the research community.[5] The biological function of m^6A modification is regulated by three types of core proteins: methyltransferases (writers), demethylases (erasers), and RNA-binding proteins (RBPs; readers), indicating a dynamic and reversible process. Abnormalities in m^6A regulators resulting in aberrant m^6A levels are associated with various diseases, such as cancer, neurological disease, and embryo retardation.[6] Moreover, accumulating evidence has shown that m^6A possesses potential clinical applications as a biomarker in the prevention, diagnosis, treatment, and prognosis of diseases.[7,8]

High-throughput sequencing for RNA methylation has revealed that m^6A is usually located in the 3′ untranslated region (3′ UTR) near the termination codon and within internal long exons,[9] which can modulate RNA fate such as processing, splicing, maturation, export, stability, translation, and degradation.[10] Also, the modification occurring near the 5′ cap can facilitate translation initiation in a cap-independent manner.[11] In addition, there is a crosslink between m^6A peaks with the 3′ UTR and binding sites of ncRNAs, suggesting a mechanism by which m^6A modification can regulate RNA transcripts through interaction with ncRNAs such as microRNAs (miRNAs).[12,13] Specifically, circular RNAs (circRNAs), a novel type of ncRNA characterized by a covalently closed loop structure, have demonstrated that they play an important role in the occurrence and development of diseases by sponging miRNAs, regulating alternative splicing, modulating parental gene expression, acting as scaffolds in protein complexes, and encoding peptides.[14,15] Recently, thousands of circRNAs derived from human tissues have attracted much attention due to their conservation, stability, and abundance in tissues and body fluids, which make them a new star in the field of liquid biopsies.[16,17] Since the specific m^6A modification of circRNAs was first reported in 2017,[18] research on m^6A circRNAs is in full swing. Zhou et al. confirmed that m^6A is widespread in circRNAs and expressed in cell-type-specific patterns, suggesting the important regulatory role of m^6A in the biological function of circRNAs.[19] Nevertheless, the interaction network between m^6A modifications and circRNAs remains unclear. In this review, we summarize the characteristics and biological functions of m^6A and circRNAs and focus on recent research progress on the interaction of m^6A modification and circRNAs. In addition, the potential clinical applications of m^6A modification and circRNAs in diagnosis and therapeutic targets are discussed.

m^6A MODIFICATION MACHINERY

As a dynamic and reversible process in eukaryotic cells, m^6A modification is catalyzed by methyltransferase and demethylase, which act

https://doi.org/10.1016/j.omtn.2022.01.007.

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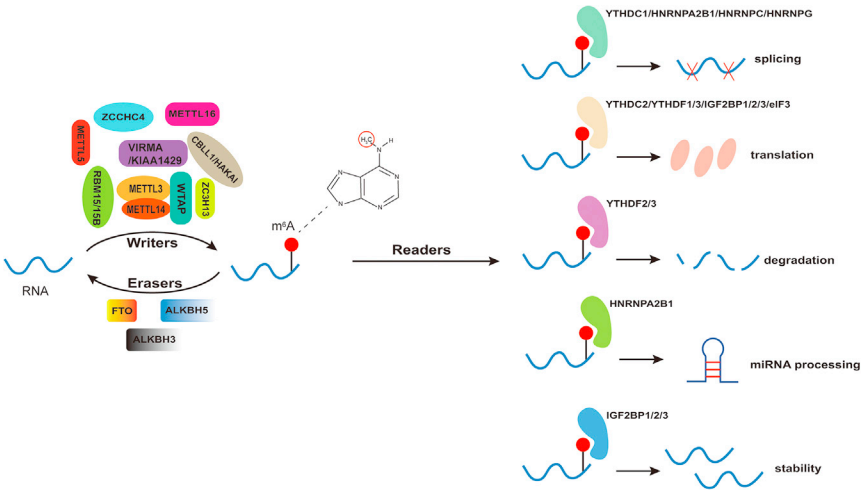

Figure 1. m^{6}A modification machinery
As a dynamic and reversible process in eukaryotic cells, m^{6}A modification is catalyzed by methyltransferase and demethylase acting as writers and erasers to add and remove m^{6}A, respectively. RNA-binding proteins called readers recognize m^{6}A sites to combinate with RNA and perform corresponding biological functions.

as writers and erasers to add and remove, respectively, m^{6}A. RBPs called readers recognize m^{6}A sites to interact with RNA and perform corresponding biological functions (Figure 1; Table 1). The dysregulation of writer, eraser, and reader proteins is involved in the pathogenesis of multiple diseases.

Methyltransferase (writers)

The m^{6}A methyltransferase complex (MTC) is usually composed of various proteins. Among the multisubunit complexes, methyltransferase-like protein 3 (METTL3) is the only core protein with catalytic activity that can transfer methyl groups from *S*-adenosyl methionine (SAM) to adenine bases of RNA transcripts through the internal SAM-binding domain. Another critical component of m^{6}A MTCs is METTL14, which also contains a SAM motif, but has no catalytic function. It acts as a binding partner of METTL3, maintaining the stability of the METTL3-14 heterodimer and recognizing the m^{6}A-specific sequence (RRACH).[20,21] In addition, many cofactors play essential roles in m^{6}A installation, including Wilms tumor 1-associated protein (WTAP), Vir-like m^{6}A methyltransferase-associated (VIRMA), RNA-binding motif protein 15/15B (RBM15/15B), and zinc finger CCCH-type containing 13 (ZC3H13). WTAP has been demonstrated to facilitate m^{6}A modification by guiding METTL3-14 heterodimer localization to nuclear spots.[22] VIRMA, also known as KIAA1429, recruits the METTL3/METTL14/WTAP complex to preferentially mediate m^{6}A modification in the 3′ UTR and near the termination codon. In addition, VIRMA can regulate alternative polyadenylation by interacting with cleavage and polyadenylation specificity factor subunit 5 (CPSF5).[23] RBM15 and RBM15B are homologous proteins and perform similar functions, which preemptively bind to the U-rich region and guide the METTL3/METTL14/WTAP complex to m^{6}A-specific motifs.[24,25] ZC3H13 combines with WTAP to promote MTC deposition in nuclear spots and enhance m^{6}A modification.[24] In addition, Cbl proto-oncogene-like 1 (CBLL1, also known as HAKAI) supports efficient methylation through the nuclear location of ZC3H13-WTAP-VIRMA-CBLL1.[26] As another zinc finger CCCH-type containing protein, ZCCHC4 has been reported to be a new m^{6}A methyltransferase for human 28S rRNA and involved in translation biology.[27] In recent years, the identification of other novel RNA methyltransferases has broadened our horizons. METTL16 is an active m^{6}A methyltransferase that targets U6 small nuclear RNA (snRNA) at position 43, which is important for U6 snRNA to recognize the 5′ splice site during precursor mRNA (pre-mRNA) splicing.[28] METTL5 is another newly discovered enzyme that catalyzes m^{6}A installation for 18S rRNA by forming a METTL5-TRMT112 heterodimeric complex to increase metabolic stability, indicating a novel RNA-binding pattern different from the complex of the METTL3-14 heterodimer.[29] Even so, there may be more m^{6}A methyltransferases to be explored further.

Demethylases (erasers)

m^{6}A demethylases function as erasers to remove m^{6}A methylation and thus maintain m^{6}A modification as a dynamic and reversible process. To date, only two m^{6}A demethylases, namely, the fat mass and obesity-associated protein (FTO) and α-ketoglutarate-dependent dioxygenase alk B homolog 5 (ALKBH5), have been widely reported. These two proteins belong to the ALKB dioxygenase family and depend on ferrous iron (Fe^{2+}) cofactor and α-ketoglutaric acid to catalyze demethylation. FTO was originally known to be an important obesity-related gene that contributed to increased body mass index and severe obesity in childhood and adults.[49,50] The initial link between FTO and m^{6}A was first discovered when it was found that FTO could exhibit efficient oxidative demethylation activity on m^{6}A residues in RNA.[30] In acute myeloid leukemia (AML), FTO plays an oncogenic role by regulating the expression of ASB2 and RARA in an m^{6}A-dependent manner.[51] However, a recent study demonstrated that in FTO knockouts, m^{6}A peaks were highly enriched in the 5′ UTR, the site of 2′-*O*-dimethyladenosine (m^{6}A$_m$), indicating that FTO preferentially demethylates m^{6}A$_m$ and controls mRNA stability.[52] Nevertheless, since the abundance of m^{6}A is much higher than that of m^{6}A$_m$, FTO mainly mediates the demethylation of m^{6}A. In addition, FTO was shown to mediate RNA demethylation of m^{1}A in transfer RNA (tRNA).[31] ALKBH5, as an FTO homolog, was the second identified demethylase protein exhibiting m^{6}A demethylase activity.[32] Unlike FTO, ALKBH5 can catalyze the removal of m^{6}A methylation without intermediate products and preferentially recognize m^{6}A for demethylation. ALKBH5-mediated m^{6}A demethylation of NANOG mRNA partly induces the breast cancer stem cell phenotype.[53] In glioblastoma, ALKBH5 maintains the

Table 1. The core regulatory proteins in m^{6}A modification

Type	Regulator	Function	Mechanism	References
Methyltransferase	METTL3	writer	transfer methyl from *S*-adenosyl methionine (SAM) to adenine bases	Wang et al.[20,21]
	METTL14	writer	maintain the stability of METTL3-14 heterodimer and recognize the m^{6}A-specific sequence (RRACH)	Wang et al.[20,21]
	WTAP	writer	facilitate m^{6}A modification by guiding METTL3-14 heterodimer localization to nuclear spots	Ping et al.[22]
	VIRMA/KIAA1429	writer	recruit METTL3/METTL14/WTAP complex in 3′ UTR and near the termination codon and regulate alternative polyadenylation	Yue et al.[23]
	RBM15/RBM15B	writer	bind to U-rich region and guide METTL3/METTL14/ WTAP complex to m^{6}A-specific motifs	Knuckles et al.,[24] Patil et al.[25]
	ZC3H13	writer	combine with WTAP to promote MTC deposition in nuclear spots and enhance m^{6}A modification	Knuckles et al.[24]
	CBLL1/HAKAI	writer	support efficient methylation through the nuclear location of ZC3H13-WTAP-VIRMA-CBLL1	Wen et al.[26]
	ZCCHC4	writer	new m^{6}A methyltransferase for human 28S rRNA; involved in translation biology	Ma et al.[27]
	METTL16	writer	target U6 small nuclear RNA (snRNA) at position 43 and recognize the 5′ splice site during pre-mRNA splicing	Warda et al.[28]
	METTL5	writer	catalyze m^{6}A installation for 18S rRNA by forming METTL5-TRMT112 heterodimeric complex to increase metabolic stability	van Tran et al.[29]
Demethylases	FTO	eraser	mediate demethylation of m^{6}A in RNA and m^{1}A in tRNA	Jia et al.,[30] Wei et al.[31]
	ALKBH5	eraser	preferentially recognize the m^{6}A for demethylation	Zheng et al.[32]
	ALKBH3	eraser	mediate the demethylation of m^{6}A, m^{1}A, and m^{3}C on tRNA	Ueda et al.,[33] Chen et al.[34]
RNA-binding proteins (RBPs)	YTHDC1	reader	regulate mRNA splicing and mediate export of m^{6}A-containing mRNA from the nucleus to the cytoplasm	Xiao et al.,[35] Roundtree et al.[36]
	YTHDC2	reader	essential for the enhancement of translation efficiency and regulation of mammalian spermatogenesis	Tanabe et al.,[37] Mao et al.,[38] Hsu et al.[39]
	YTHDF1	reader	mediate translation promotion via the interaction with translation initiation complex	Wang et al.[40]
	YTHDF2	reader	induce the instability and accelerate the degradation of m^{6}A-methylated mRNA through the interaction with P bodies	Wang et al.,[41] Batista et al.,[42] Geula et al.[43]
	YTHDF3	reader	facilitates translation and affects the decay of m^{6}A-containing mRNA in synergy with YTHDF1 and YTHDF2, respectively	Shi et al.[44]
	HNRNPA2B1	reader	bind m^{6}A-bearing RNAs to elicit alternative splicing effects and interact with the microRNA (miRNA) microprocessor complex protein DGCR8 to promote primary miRNA processing	Alarcon et al.[45]
	HNRNPC	reader	affect the abundance and alternative splicing of target RNAs	Liu et al.[46,47]
	HNRNPG	reader	affect the abundance and alternative splicing of target RNAs	Liu et al.[46,47]
	IGF2BP1/2/3	reader	promote mRNA stability and translation in an m^{6}A-dependent manner	Huang et al.[48]
	eIF3	reader	bind to the 5′ UTR of m^{6}A-bearing mRNA and recruit the 43S ribosomal complex to initiate translation in a cap-independent manner	Meyer et al.,[11] Huang et al.[48]

tumorigenicity of stem-like cells by sustaining FOXM1 expression and the cell proliferation program.[54] Recently, ALKBH3 was identified as a novel demethylase protein that preferentially mediates the demethylation of m^6A, m^1A, and 3-methylcytidine (m^3C) on tRNA.[33,34]

RBPs (readers)

m^6A "readers," a group of RBPs that can recognize m^6A modifications, contribute to different biological functions of target RNAs. At present, there are three categories of readers that have been extensively studied, including YT521-B homology (YTH) domain family proteins, heterogeneous nuclear ribonucleoproteins (HNRNP) family and insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs). YTH domain-containing proteins have 5 members: YTH domain containing 1 (YTHDC1), YTH domain containing 2 (YTHDC2), YTH domain family protein 1 (YTHDF1), YTH domain family protein 2 (YTHDF2), and YTH domain family protein 3 (YTHDF3). Of them, YTHDC1 is nuclear enriched and located in nuclear speckles, which can regulate mRNA splicing by recruiting pre-mRNA splicing factors to the binding sites of target RNAs.[35] In addition, YTHDC1 mediates the export of m^6A-containing mRNA from the nucleus to the cytoplasm by interacting with splicing factors and nuclear export adaptor proteins.[36] In the cytoplasm, YTHDC2 recognizes a smaller number of m^6A sites, whereas the DF family (YTHDF1–3) can bind all m^6A sites in RNA.[25] YTHDC2 is essential for the enhancement of translation efficiency[37,38] and regulation of mammalian spermatogenesis.[39] YTHDF1 was shown to mediate translation promotion via interactions with translation initiation complexes, such as eukaryotic translation initiation factor 3 (eIF3), 4E (eIF4E), 4G (eIF4G), poly (A) binding protein (PABP), and the 40S ribosomal subunit.[40] YTHDF2, the most abundant in nearly all types of cells, has been identified to induce instability and accelerate the degradation of m^6A-methylated mRNA through interaction with P bodies.[41–43] YTHDF3 facilitates translation and affects the decay of m^6A-containing mRNA in synergy with YTHDF1 and YTHDF2.[44] Members of the HNRNP family, including HNRNPA2B1, HNRNPC, and HNRNPG, are located in the nucleus. HNRNPA2B1 can bind m^6A-bearing RNAs to elicit alternative splicing effects and interact with the miRNA microprocessor complex protein DGCR8 to promote primary miRNA processing.[45] Unlike HNRNPA2B1, HNRNPC and HNRNPG cannot directly bind to the sites of m^6A. However, due to changes in the local structure of RNA caused by m^6A, the binding activities of HNRNPC and HNRNPG are facilitated, thereby affecting the abundance and alternative splicing of target RNAs.[46,47] As a distinct family of m^6A readers, IGF2BP proteins, including IGF2BP1/2/3, affect gene expression output by promoting mRNA stability and translation in an m^6A-dependent manner.[48] Moreover, eIF3 has been identified as a novel m^6A reader, with the evidence suggesting that it preferentially crosslinks to m^6A-bearing mRNA.[11] Moreover, eIF3 has been proposed to directly bind to the 5′ UTR of m^6A-bearing mRNA and recruit the 43S ribosomal complex to initiate translation in a cap-independent manner.[48]

circRNA OVERVIEW

circRNAs are a special kind of RNA, with a covalently closed, single-stranded structure produced from pre-mRNA back-splicing by distinct mechanisms. A number of studies have shown that circRNAs possess many unique characteristics, such as high stability, abundance, species conservation, and tissue- and disease-specific expression patterns, which enable them to perform crucial biological functions.[15]

The biogenesis and properties of circRNAs

circRNAs were thought to be the result of alternative splicing of pre-mRNA through various mechanisms (Figure 2). Most currently studied circRNAs are generated by back-splicing of exons, in which a downstream 5′ splice donor site is ligated with an upstream 3′ splice acceptor site. According to the different combinations of sequences and domains, circRNAs can be divided into three types: exonic circRNAs (EciRNAs), which originated from exon sequence only;[55,56] intronic circRNAs (CiRNAs), which contain intron sequences only;[57] and exon-intron circRNAs (EIciRNAs), which consist of exon and intron sequences.[58] Two well-known models of EciRNA and EIciRNA biogenesis were proposed by Jeck et al., called lariat-driven circularization and intron-pairing-driven circularization.[59] CiRNAs are derived from lariat introns containing a 7-nt GU-rich element near the 5′ splice site and an 11-nt C-rich element at the branchpoint site.[57] Recently, some special forms of circRNAs have been identified. Studies have reported that transcribed exons of different genes influenced by translocations could give rise to fusion circRNAs (f-circRNAs), which often function as drivers of tumorigenesis.[60–62] Exon sequencing revealed that readthrough circRNAs (rt-circRNAs) originate from exons in different genes.[14,63] In addition, mitochondrial genome-encoded circRNAs (mecciRNAs) play important roles in the mitochondrial entry of proteins.[64]

With the rapid development of next-generation RNA sequencing (RNA-seq) of non-polyadenylated transcriptomes, circRNAs have been extensively investigated and found to abundantly exist in eukaryotic cells.[65] In general, EciRNAs contain <5 exons, with the splice lengths ranging from 100 to 4,000 nt.[66] Hundreds of human genes give rise to many circRNAs, and the same host gene could produce many different circRNAs due to alternative circularization, which enriches the diversity of circRNAs.[55,67] Although the abundance of most circRNAs is less than that of the corresponding linear RNAs, under some circumstances, circRNA expression is dozens of times greater than that of the linear transcripts.[59] circRNAs have high stability because of their special covalently closed structure without a polyadenylated tail. According to recent reports, circRNAs can remain resistant to RNA exonucleases and have a longer average half-life than their linear RNAs.[68–71] Although the detailed mechanisms of circRNA degradation have not been elucidated, circRNAs contain miRNA response elements (MREs), which could lead to circRNA degradation by interacting with the AGO protein and forming an RNA-induced silencing complex (RISC).[72] Moreover, Park et al. demonstrated that m^6A is involved in the degradation process of

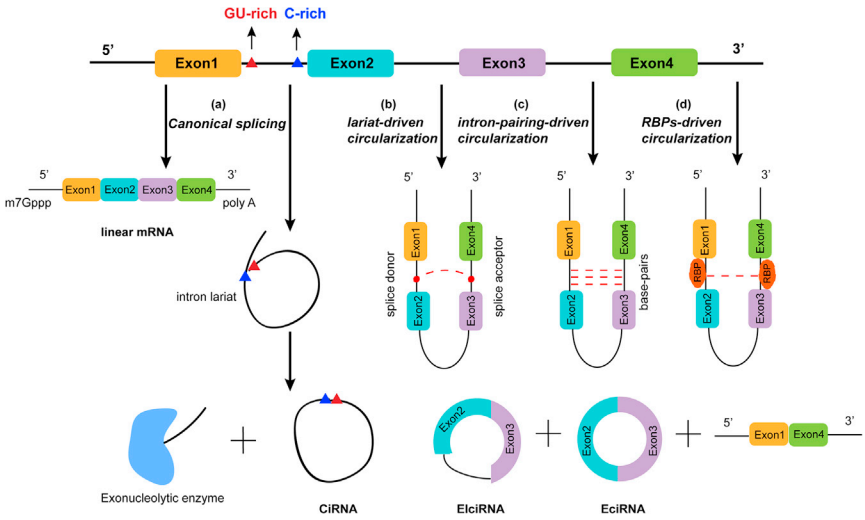

Figure 2. The models of circRNAs biogenesis

(A) Canonical splicing: The GU-rich element near the 5′ splice site and C-rich element close to the branchpoint site were ligated to form ciRNAs. (B) Lariat-driven circularization: The 5′ splice donor site of exon 4 covalently binds to the 3′ splice acceptor site of exon 1 to form lariat structure and produce ElciRNAs or EciRNAs. (C) Intron-pairing-driven circularization: The complementary base pairs between introns bring splicing sites close to form ElciRNAs or EciRNAs. (D) RBP-driven circularization: RBPs bridge distal splice site to facilitate cyclization.

circRNAs.[73] Typically, circRNAs are evolutionarily conserved among different species.[74] For instance, 2,121 circRNAs identified in human fibroblasts can be matched to the mouse genome.[59] This phenomenon may result from the conservation of splicing regulatory elements and complementary flanking introns such as *Alu* elements. Furthermore, the expression pattern of circRNAs is cell type specific and occurs in a spatiotemporal manner.[75,76]

The regulation of circRNA generation depends on several kinds of factors, including spliceosomes, *cis*-complementary sequences in flanking introns, and special proteins.[77] The spliceosome can recognize specific splice sites of the exon flanks, promoting circRNAs biogenesis.[78] Back-splicing of circRNA formation is boosted by intronic complementary sequences (ICSs), which are usually mediated by complementary inverted-repeat *Alu* elements and non-repetitive complementary sequences in human cells.[55,59,79] The competition of RNA pairs in the same intron, flanking introns, and different sets of introns significantly influence the efficiency of circRNA production.[80–82] In addition, a number of RBPs regulate the back-splicing of circRNA biogenesis in *trans*. In one mode, RBPs bridge distal splice sites to facilitate cyclization. The splicing factor muscleblind (Mbl) can modulate circMbl biosynthesis by directly binding to conserved Mbl binding sites in flanking introns.[81] Protein quaking (QKI), as an alternative splicing factor, dynamically regulates the production of abundant circRNAs during the epithelial-mesenchymal transition (EMT) process, depending on the QKI recognition motif in introns, which could make splice sites closer together, benefiting the back-splicing of circRNAs.[83] Another mode is that RBPs bind to ICSs and stabilize RNA pairs in intron flanks via double-stranded RNA (dsRNA)-binding domains (dsRBDs). For example, the nuclear factor 90 (NF90) and NF110 containing 2 dsRBDs can bind to intronic inverted-repeat *Alu* elements and promote circRNA formation.[84] However, DHX9, a nuclear RNA helicase containing a dsRBD and an RNA helicase domain, can dampen circRNA formation by binding to intronic inverted-repeat *Alu* elements and disassembling RNA pairs in intron flanks using RNA helicase activity.[85] Adenosine deaminase acting on RNA 1 (ADAR1), a kind of RNA-editing enzyme, performs A-to-I editing of inverted-repeat *Alu* elements, impairing the stability of RNA pairs and negatively regulating circRNA expression.[86,87] In recent years, a novel role for m^{6}A in the biogenesis of circRNAs has been uncovered.[88]

The regulatory mechanisms of circRNAs

Emerging studies have revealed that circRNAs participate in the pathophysiological processes through various regulatory mechanisms at multiple levels. Notably, circRNAs have been reported to serve as miRNA decoys, protein scaffolds, alternative splicing regulators, and translation templates. circRNAs in the cytoplasm may directly bind to miRNAs via complementary miRNA binding sites to regulate downstream target genes. Despite the concerns that most circRNAs possess <10 miRNA binding sites, the role of circRNAs as competing endogenous RNAs (ceRNAs) has still been widely investigated.[89,90] Accumulating evidence has demonstrated that miRNA sponging may be the most common mode of action of circRNAs. Apart from interacting with miRNAs, circRNAs can engage with different proteins and function as protein scaffolds. For example, CUT-like homeobox 1 (CUX1)-generated circular RNA circ-CUX1 contributes to aerobic glycolysis and neuroblastoma progression by binding to EWS RBP 1 (EWSR1) to facilitate MYC-associated zinc finger protein (MAZ) transactivation, resulting in transcriptional alteration of CUX1 and other genes associated with tumor progression.[91]

circRNAs in the nucleus have distinct functions, such as regulation of transcription and alternative splicing. Typically, EIciRNAs can enhance parental gene transcription through interaction with U1 snRNA and RNA polymerase II (RNA Pol II) transcription complex at the promoters of genes.[58] The splicing factor Mbl can modulate circMbl biosynthesis by directly binding to conserved Mbl binding sites in flanking introns, which indicates that circRNA production competes with pre-mRNA splicing.[81] Recently, research on the function of circRNAs translation has drawn much attention since it was first reported that endogenous circRNAs have the ability to translate proteins or peptides.[92] van Heesch et al. have identified hundreds of small peptides translated from lncRNAs and circRNAs by analyzing the translatomes of 80 human hearts and validated the protein

products *in vivo*, supporting the fact that circRNAs can serve as the potential translation templates of proteins.[93] To date, the mechanisms of circRNAs translation are internal ribosome entry site (IRES)-driven and m^{6}A-mediated initiation.[94]

The biological functions of circRNAs

A growing number of circRNAs have been implicated in the initiation and development of various diseases, especially tumors. The functions of circRNAs can be mainly divided into oncogenic and antioncogenic. For example, by adopting ultra-deep RNA-seq and short hairpin RNA (shRNA)-screening libraries, Chen et al. identified a total of 171 circRNAs essential for prostate cancer cell proliferation, whereas their host linear transcripts were not essential.[95] This phenomenon reflected that the function of circRNAs may be independent of their parental linear RNAs. However, circYap could enhance apoptosis and restrain the proliferation and metastasis of cancer cells by binding with the translation initiation-associated proteins eIF4G and PABP to antagonize YAP translation.[96] In addition, Epstein-Barr virus (EBV)-encoded circLMP2A plays crucial roles in inducing and maintaining stemness phenotypes in EBV-associated gastric cancer. Impaired circLMP2A expression can suppress cell growth and metastasis *in vitro* and *in vivo*.[97] These studies indicate that different types of circRNAs influence cell transformation properties in various ways.

The roles of circRNAs have been widely investigated in other non-tumor diseases. It has been shown that circRNAs are abundant and conserved in mammalian neurons and synaptogenesis, suggesting that circRNAs play vital roles in regulating neuronal and synaptic functions. Loss of the CDR1as locus in the mouse genome affects synaptic transmission and causes neuropsychiatric disorders.[98] In cardiovascular diseases, circFndc3b overexpression in cardiac endothelial cells enhances neovascularization, reduces cardiomyocytes and endothelial cell apoptosis, and improves myocardial functions.[99] In adipose biology, Arcinas et al. discovered that circTshz2-1 and circArhgap5-2 serve as indispensable regulators in adipocyte differentiation and metabolism.[100]

Recently, emerging roles of circRNAs in the immune system have been uncovered. On the one hand, circRNAs have been shown to exhibit cell-type-specific expression in different types of immune cells and induce their differentiation.[101,102] On the other hand, circRNAs can regulate the activity of innate immune cells and then facilitate disease development.[103] In the absence of viral infection, endogenous circRNAs usually bind to innate immunity proteins and inactivate immunity. Upon infection, these proteins are released to initiate the antiviral immune response by recognizing viral nucleic acids, and the level of endogenous circRNAs globally decreases. As mentioned above, the NF90/NF110 complex can bind to intronic inverted-repeat *Alu* elements and promote circRNAs formation in the nucleus.[84] circRNAs in the cytoplasm can also interact with the NF90/NF110 complex. Once viral infection occurs, nuclear NF90/NF110 rapidly translocates to the cytoplasm to inhibit the translation of viral mRNAs,[104] leading to a global reduction in circRNA production. Meanwhile, NF90/NF110 are separated from cytoplasmic circRNAs to interact with viral transcripts and block virus replication.[84] In addition, endogenous circRNAs can bind to antiviral protein kinase R (PKR) to prevent innate immune activation. Viral infection triggers the degradation of circRNAs to free PKR and stimulates innate immunity.[105] However, foreign circRNAs have opposite effects to endogenous circRNAs. Engineered foreign circRNAs transfected into cells can stimulate innate immunity and inhibit viral infection by activating immune genes such as PKR, melanoma-differentiation-associated gene 5 (MDA5), and retinoic-acid-inducible gene-I (RIG-I).[106] Of note, m^{6}A-modified circRNAs also have important roles in innate immunity.

CHARACTERISTICS OF m^{6}A circRNAs

Previous studies have shown that RNase R-resistant (non-linear) RNAs contain a strong m^{6}A signal, suggesting that circRNAs may be modified by m^{6}A.[19] METTL3/METTL14 complex is required for the m^{6}A modification of mRNAs, which is also essential for m^{6}A circRNAs. The depletion of METTL3 or METTL14 results in the reduced m^{6}A modification of circRNA-enriched RNA, which is more significant with combined METTL3/METTL14 depletion.[19] Similarly, m^{6}A circRNAs are read by the same proteins, such as YTHDF1 and YTHDF2, which interact with mRNAs. YTHDF2 is widely known to regulate the stability of mRNAs by forming a complex with m^{6}A mRNAs.[41] However, YTHDF2, which is involved in m^{6}A circRNAs, does not appear to promote the degradation of circRNAs. An interesting phenomenon was observed in which mRNAs methylated on the same exons encoded by the parental genes of m^{6}A circRNAs had the shortest half-lives among all m^{6}A mRNAs, showing that the stability of mRNAs is regulated by YTHDF2 in a process involving the recognition of m^{6}A circRNAs. Furthermore, the m^{6}A circRNA levels are variable across different species and similar to mRNAs in distribution.

Although m^{6}A modification is widespread in circRNAs, unique patterns that are distinct from mRNAs are exhibited. First, m^{6}A circRNAs and m^{6}A peaks in mRNAs across genes have different distributions. m^{6}A sites in mRNAs are most common in the region from the end of the coding sequence to the 3′ end (3′ UTR), but exons encoding m^{6}A circRNAs are often located in the region from the transcription start site (TSS) to the start of the coding sequence (5′ UTR). Thus, the majority of m^{6}A circRNAs are generated from exons without m^{6}A peaks in mRNAs. Second, numerous m^{6}A circRNAs are expressed in a cell-type-specific manner, even though their parental genes or circRNAs are expressed in both cell types, indicating that m^{6}A circRNAs are involved in different biological processes in specific cell types. Third, m^{6}A methylation is more abundant in circRNAs composed of long single exons than multi-exon m^{6}A circRNAs. In addition, transposable elements (TEs) are significantly enriched in the flanking regions of m^{6}A circRNAs, suggesting that the density of TEs flanking circRNAs is associated with m^{6}A modification.

ROLES OF m^{6}A METHYLATION ON circRNAs

Accumulating studies have identified that m^{6}A modifications have various regulatory effects on circRNAs, including their biogenesis,

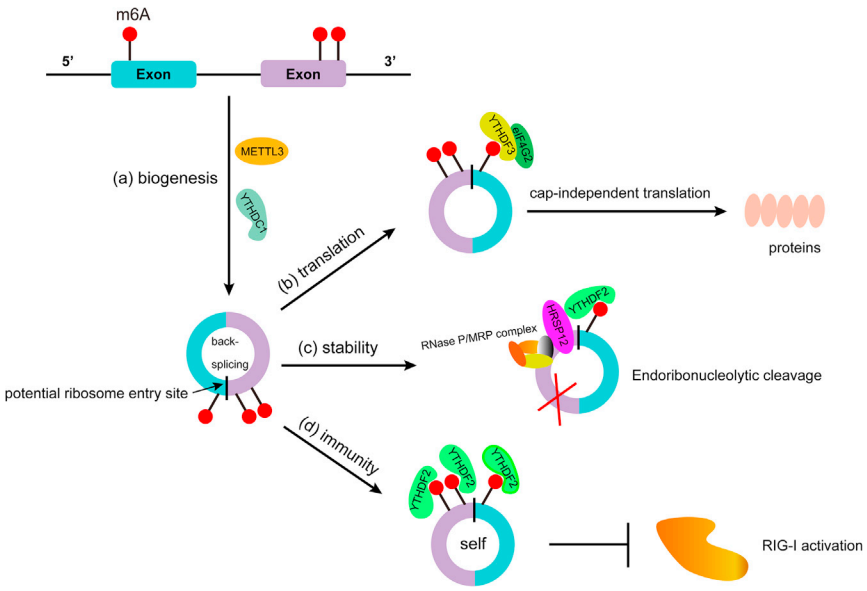

Figure 3. Roles of m^{6}A methylation on circRNAs
(A) circRNA production correlates with m^{6}A levels and is modulated by ALKBH5 and METTL3. (B) m^{6}A residues can drive efficient initiation of protein translation from circRNAs in a cap-independent manner. (C) m^{6}A-containing circRNAs are endoribonucleolytically cleaved by YTHDF2-HRSP12-RNase P/MRP complex. (D) m^{6}A suppression of circRNA immunity was mediated by YTHDF2, which can block endogenous circRNAs from activating the RIG-I antiviral pathway.

splicing, localization, transport, and degradation (Figure 3). Here, we describe the roles of m^{6}A modifications in the regulation and function of circRNAs (Table 2).

m^{6}A methylation modulates the biogenesis of circRNAs

Recently, the role of m^{6}A in the biogenesis of circRNAs has been investigated. Studies have reported that circRNAs are abundantly synthesized in male germ cells during spermatogenesis.[88] Junction points of these circRNAs appear to occur in m^{6}A-enriched sites, which are usually located around the start and stop codons of linear mRNAs.[123] This evidence implies that circRNA production correlates with m^{6}A levels, which are modulated by ALKBH5 and METTL3.[88] In addition, half of these circRNAs contain large open reading frames (ORFs) with m^{6}A-modified start codons and have coding potential, revealing a novel mechanism by which circRNAs can maintain the continuous and stable production of proteins in the absence of linear transcripts.[88] Another study discovered that the biogenesis of circ-ZNF609 was modulated by METTL3 and YTHDC1, which displayed specific m^{6}A signatures that controlled its accumulation.[107] This feature is shared with other circRNAs because of a significant direct correlation with METTL3 requirement, YTHDC1 binding, and the ability of m^{6}A exons to undergo circularization.

The transcriptome-wide map of m^{6}A circRNAs in hypoxia-mediated pulmonary hypertension (HPH) showed that m^{6}A circRNAs were derived mainly from genes spanning single exons in the control and HPH groups. The expression of m^{6}A-circRNAs tended to be reduced in comparison with that of non-m^{6}A circRNAs in HPH, suggesting that m^{6}A methylation could influence the expression of circRNAs.[124] This characteristic was also observed in lens epithelium cells from age-related cataracts.[125] In addition, Guo et al. found that the decreased expression of hsa_circ_0029589 through elevated m^{6}A levels promoted by METTL3 may facilitate macrophage pyroptosis in acute coronary syndrome (ACS) and atherosclerosis (AS).[108] The expression of METTL3 upregulated by HBx proteins in hepatitis B virus-associated hepatocellular carcinoma increased the level of m^{6}A circ-ARL3, which YTHDC1 bound to promote its reverse splicing and biogenesis.[109] Also, METTL3-induced circ1662 expression by binding its flanking sequences and installing m^{6}A modifications could promote colorectal carcinoma (CRC) metastasis by accelerating YAP1 nuclear transport.[110] As the host gene of circMETTL3, METTL3 may regulate its expression in an m^{6}A-dependent manner to facilitate breast cancer progression.[111] In addition, Rong et al. illustrated that METTL3 facilitated the m^{6}A modification process and controlled the accumulation of circHPS5.[112] The nucleus-cytoplasm output of circHPS5 could be mediated by recruiting YTHDC1, which promoted the EMT and cancer stem-like cell (CSC) phenotypes in hepatocellular carcinoma (HCC).[112] Furthermore, as a key component of the m^{6}A methyltransferase complex, KIAA1429 could regulate the expression of circDLC1, contributing to the inhibition of HCC progression.[113] These results suggest that m^{6}A methylation affects the biogenesis and expression of circRNAs in different ways.

m^{6}A methylation regulates the stability of circRNAs

circRNAs remain resistant to RNA exonuclease and have a longer average half-life than linear RNAs due to their special covalently closed structure without a polyadenylated tail.[68–71] However, the detailed mechanisms of circRNAs degradation have not been elucidated. A previous study reported that circRNAs with miRNA response elements (MREs) could lead to their degradation by interacting with the AGO protein and forming RISC.[72] Recently, a new mechanism was uncovered in which m^{6}A-containing linear RNAs and circRNAs are endoribonucleolytically cleaved by the YTHDF2-HRSP12-RNase P/MRP (mitochondrial RNA processing) complex.[73] HRSP12 acts as a bridge to link YTHDF2 and RNase P/MRP. HRSP12 and RNase P/MRP can destabilize YTHDF2-bound transcripts. circRNAs associated with YTHDF2 in a HRSP12-dependent manner are preferentially reduced by RNase P/MRP. Thus, these findings demonstrate that m^{6}A is involved in the degradation process of circRNAs.

m^{6}A modification has also been considered to elevate the expression of circRNAs, resulting partially from increased RNA stability. For

Table 2. Roles of m^{6}A methylation on circRNAs

m^{6}A regulators	circRNA	m^{6}A levels	Effects on circRNAs	Diseases	References
ALKBH5/METTL3	a subset of circRNAs	decrease/increase	modulate the biogenesis	spermatogenesis	Tang et al.[88]
METTL3/YTHDC1	circ-ZNF609	increase	modulate the biogenesis	rhabdomyosarcoma tumors	Di Timoteo et al.[107]
METTL3	hsa_circ_0029589	increase	decrease its expression	ACS, AS	Guo et al.[108]
METTL3/YTHDC1	circ-ARL3	increase	promote splicing and biogenesis	HCC	Rao et al.[109]
METTL3	circ1662	increase	induce its expression	CRC	Chen et al.[110]
METTL3	circMETTL3	increase	regulate its expression	breast cancer	Li et al.[111]
METTL3/YTHDC1	circHPS5	increase	promote biogenesis and nucleus-cytoplasm output	HCC	Rong et al.[112]
KIAA1429	circDLC1	increase	regulate its expression	HCC	Liu et al.[113]
METTL3/FTO/YTHDF1/2	circRNA-SORE	increase	enhance its stability	HCC	Xu et al.[114]
METTL3	circCUX1	increase	stabilize its expression	hypopharyngeal squamous cell carcinoma	Wu et al.[115]
METTL3	circ0000069	increase	improve its stability	cervical cancer	Chen et al.[116]
YTHDF3/eIF4G2	circ-ZNF609	recognition	increase translation	HeLa cells	Di Timoteo et al.[107]
METTL3/METTL14	circE7	increase	facilitate translation	human papillomavirus	Zhao et al.[117]
FTO	circRNA ARHGAP35	decrease	promote translation	HCC	Li et al.[118]
YTHDF2	circRNA	recognition	suppress immunity	mammalian cells	Chen et al.[119]
YTHDC1	circNSUN2	recognition	export to cytoplasm from nucleus	CRC	Chen et al.[120]
METTL14	circGFRα1	increase	export to cytoplasm from nucleus	FGSCs	Li et al.[121]
METTL3/METTL14	circNDUFB2	increase	impair interaction with proteins	NSCLC	Li et al.[122]

example, m^{6}A methylation enhanced the stability of circRNA-SORE, which was upregulated in sorafenib-resistant HCC cells and induced sorafenib resistance.[114] METTL3-mediated m^{6}A modification stabilized the expression of circCUX1, which conferred radioresistance in hypopharyngeal squamous cell carcinoma.[115] m^{6}A modification improved circ0000069 transcript stability and promoted cervical cancer cell proliferation and migration.[116] These studies hint that m^{6}A methylation could regulate the stability of circRNAs to affect their biological functions.

m^{6}A methylation facilitates the translation of circRNAs

Due to the lack of a 5′ 7-methylguanosine cap structure and poly(A) tail, two factors that are required for mRNAs translation, circRNAs have been classified as ncRNAs without the ability to encode proteins. However, accumulated evidence suggests that circRNAs may be used as templates for protein synthesis. Artificial circRNAs can be translated in a cap-independent manner through IRES elements directly binding initiation factors or the ribosome.[126,127] Subsequently, Yang et al. discovered that m^{6}A residues are abundant in circRNAs and can drive efficient initiation of protein translation from circRNAs.[18] The m^{6}A-induced translation of circRNAs could be increased by various cellular stresses, such as heat shock or upregulation of the methyltransferase METTL3/14, but could be decreased by the demethylase FTO.[18] In addition, the eukaryotic translation initiation factor eIF4G2 and the m^{6}A reader YTHDF3 are essential for circRNA translation driven by m^{6}A, with a possible role of YTHDF3 in recruiting eIF4G2 to the m^{6}A-containing RNA.[18] Bozzoni et al. identified that circ-ZNF609 translation is modulated through recognition by YTHDF3 and eIF4G2.[107] Overexpression of either of these two factors resulted in the increased translation of circ-ZNF609. YTHDF3 and eIF4G2 were physically associated with endogenous circ-ZNF609, indicating the relevance of both of these factors in the translational control of circ-ZNF609.[107]

circE7, derived from human papillomavirus 16 (HPV16), is associated with polysomes and translated to produce the E7 oncoprotein. Zhao et al. found that circE7 possessed multiple potential m^{6}A sites and was confirmed to be methylated.[117] Knockdown of METTL3/14 resulted in a decrease in circE7 expression after m^{6}A immunoprecipitation. Mutation of the m^{6}A motifs also significantly decreased the abundance of circE7 and inhibited E7 oncoprotein expression.[117] These results indicated that m^{6}A modification facilitates the cap-independent translation of circE7. Similarly, a novel circRNA ARHGAP35 promotes cancer cell progression through translation into an oncogenic protein driven by m^{6}A modification. Overexpression of the m^{6}A demethylase FTO significantly reduced the abundance of m^{6}A and translation of the oncogenic protein, confirming that m^{6}A modification is essential for the efficient protein translation from circARHGAP35.[118]

m^{6}A modification controls circRNA immunity

circRNAs have important biological roles in immune cells and immune response.[128] A recent study revealed that in the absence of viral infection, endogenous circRNAs possessing 16- to 26-bp imperfect

Table 3. Roles of circRNAs in the regulation of m^{6}A modification

Role mode	circRNA	m^{6}A regulators	m^{6}A levels	Function	Diseases	References
Combined with m^{6}A regulators	circSTAG1	ALKBH5	increase	promote the degradation of FAAH	depressive disorder	Huang et al.[129]
	circZbtb20	ALKBH5	decrease	improve *Nr4a1* stability	ILC3 homeostasis	Liu et al.[130]
	circNOTCH1	METTL14	decrease	maintain NOTCH1 stability	NSCLC	Shen et al.[131]
	circPTPRA	IGF2BP1	block recognition of m^{6}A	downregulate the stability of FSCN1 and MYC	bladder cancer	Xie et al.[132]
	circ0008399	WTAP	increase	upregulate TNFAIP3 expression	bladder cancer	Wei et al.[133]
Directly regulate m^{6}A regulators	circRAB11FIP1	FTO	decrease	alter ATG5 and ATG7 expression	ovarian cancer	Zhang et al.[134]
Indirectly regulate m^{6}A regulators	circ_0072083	ALKBH5	decrease	increase NANOG stability	glioma	Ding et al.[135]
	circMAP2K4	YTHDF1	affect recognition of m^{6}A	–	HCC	Chi et al.[136]

RNA duplexes could interact with PKR monomers to suppress their activity. Upon viral infection, endonuclease RNase L degrades endogenous circRNAs to release PKR to initiate the innate immune response.[105] These findings show that endogenous circRNAs function as suppressors of immune stimulation. In contrast, exogenous circRNAs potently induce immune signaling by interacting with RIG-I in the presence of K63-polyubiquitin to cause mitochondrial antiviral signaling (MAVS) filamentation, IRF3 dimerization, and interferon production.[106,119] In addition, exogenous circRNAs could induce antigen-specific T and B cell activation, antibody production, and antitumor immunity *in vivo*.[119]

The different roles of endogenous and exogenous circRNAs in immune signaling are partly dependent on m^{6}A modification.[119] Yan et al. identified that endogenous circRNAs marked by m^{6}A modification could escape immunological surveillance and avoid eliciting an immune response.[128] They also demonstrated that m^{6}A suppression of circRNA immunity was mediated by the RBP YTHDF2, which could recruit m^{6}A-modified RNAs into phase-separated condensates via their N-terminal disordered domains and block endogenous circRNAs from activating the RIG-I antiviral pathway.[128] Collectively, this strong evidence suggests that m^{6}A modification is a key regulator of circRNAs immunity.

m^{6}A methylation affects the biological functions of circRNAs

The effects of m^{6}A modification on the biological functions of circRNAs are also reflected in other aspects. Chen et al. identified that an m^{6}A-modified circNSUN2 was elevated in CRC patients with liver metastasis and predicted poorer survival.[120] The nucleus export to the cytoplasm of circNSUN2 was mediated by YTHDC1 in an m^{6}A-dependent manner. Increased cytoplasmic circNSUN2 stabilized high-mobility group AT-hook (*HMGA2*) mRNA and interacted with IGF2BP2 to promote colorectal liver metastasis.[120] In the same way, m^{6}A-modified circGFRα1 (glial cell line-derived neurotrophic factor [GDNF] receptor α1) was highly abundant in female germline stem cells (FGSCs). The m^{6}A writer METTL14 facilitated circGFRα1 export to the cytoplasm from the nucleus in an m^{6}A-dependent manner. Cytoplasmic circGFRα1 sponges miR-449 to enhance GFRα1 expression and activate the GDNF signaling pathway, ultimately promoting FGSC self-renewal.[121] In addition, Li et al. revealed that circNDUFB2 (NADH:ubiquinone oxidoreductase subunit B2) inhibits the growth and metastasis of non-small cell lung carcinoma (NSCLC) cells via a mechanism in which the TRIM25/circNDUFB2/IGF2BPs ternary complex facilitates the ubiquitination and degradation of IGF2BPs.[122] In this study, m^{6}A modification was abundant in circNDUFB2, and the interactions between circNDUFB2 and IGF2BPs were significantly impaired, with a reduction in m^{6}A levels caused by METTL3/14 knockdown.[122] Clearly, these investigations demonstrate that m^{6}A modification plays a key role in the biological functions of circRNAs.

ROLES OF circRNAs IN THE REGULATION OF m^{6}A MODIFICATION

Studies have shown that circRNAs can affect m^{6}A modification by regulating m^{6}A regulators (Table 3). However, circRNAs competitively bind to m^{6}A regulators to affect m^{6}A levels. For example, circSTAG1 was significantly downregulated in the peripheral blood of patients with major depressive disorder. Overexpressed circSTAG1 captured the demethylase ALKBH5, which increased m^{6}A levels of fatty acid amide hydrolase (FAAH) mRNA by reducing the translocation of ALKBH5 into the nucleus and promoted the degradation of FAAH in astrocytes with subsequent attenuation of depressive-like behaviors.[129] Liu et al. reported that circZbtb20 was highly expressed in group 3 innate lymphoid cells (ILC3s) and was required for their function.[130] circZbtb20 enhances the interaction of ALKBH5 with *Nr4a1* mRNA, leading to a reduction in the m^{6}A modification of *Nr4a1* mRNA to improve its stability. Subsequently, Nr4a1 initiates Notch2 signaling activation to maintain ILC3 homeostasis.[130] In NSCLC, circNOTCH1 was upregulated and competed with NOTCH1 mRNA for METTL14 binding, causing decreased m^{6}A methylation on NOTCH1 mRNA and elevating its expression by maintaining stability.[131] circPTPRA is an important tumor suppressor in bladder cancer that abolishes the promotion of cell proliferation and metastasis induced by IGF2BP1. Ectopic expression of circPTPRA could downregulate the stability of FSCN1 and MYC mRNA through interaction with KH domains of IGF2BP1 and blocking its

recognition of downstream m^{6}A-modified mRNAs.[132] In bladder cancer, circ0008399 bound with WTAP to promote the formation of the WTAP/METTL3/METTL14 m^{6}A methyltransferase complex, which upregulated the expression of tumor necrosis factor-α (TNF-α)-induced protein 3 (TNFAIP3) by increasing its mRNA stability in an m^{6}A-dependent manner.[133] However, circRNAs directly regulate the expression of m^{6}A regulators to modulate m^{6}A levels. circRAB11FIP1, overexpressed in ovarian cancer, directly bound to FTO mRNA and promoted its expression, which decreased the m^{6}A levels of ATG5 and ATG7 mRNA and altered their expression.[134] In addition, circRNA could act as an miRNAs sponge to indirectly modulate m^{6}A regulators and methylation. For instance, circ_0072083 interacts with miR-1252-5p to upregulate demethylase ALKBH5 expression, which increases NANOG mRNA stability by reducing m^{6}A levels of NANOG mRNA, leading to enhanced temozolomide resistance in glioma.[135] Chi et al. confirmed that circMAP2K4 could act as miR-139-5p sponge to upregulate the expression and activity of YTHDF1 and promote HCC proliferation.[136] These findings enrich our understanding of the roles of RNA epigenetic regulation patterns.

LIMITATIONS OF m^{6}A-MODIFIED circRNAs

Although current research on m^{6}A-modified RNAs is in full swing, our understanding is only beginning, especially regarding m^{6}A-modified circRNAs. There are many technical obstacles and challenges in studying circRNA-specific m^{6}A modifications. First, it is difficult and complicated to accurately detect the m^{6}A levels of specific circRNAs in disease-specific tissue samples and body fluids due to their low abundance, which hinders their clinical application. Second, the detection methods must be improved to precisely identify the specific sites of m^{6}A-modified circRNAs, which is beneficial in exploring their regulatory mechanisms and biological functions in diseases. Third, m^{6}A modification mediates the different roles of endogenous and exogenous circRNAs in immune signaling. More advanced technologies need to be developed to apply m^{6}A-modified circRNAs to the immunotherapy of diseases.

CONCLUSIONS AND PROSPECTS

In this review, we comprehensively summarized the characteristics and biological functions of m^{6}A and circRNAs and focused on recent advances in the interactive effects of m^{6}A modification and circRNAs, which provides a new perspective for understanding the complex regulatory network between RNA transcriptomics and epigenetic modifications.

Following the rapid development of biological methods and informatics technologies, numerous circRNAs have been identified to stably exist in body fluids, such as serum, plasma, and urine.[137] Due to their high stability and conservation, circRNAs are ideal candidates for liquid biopsy biomarkers in many human diseases, such as cancers, autoimmune diseases, and cardiovascular diseases.[138] The specific circRNAs activated by m^{6}A present differential expression in special tissues and developmental stages. It is possible that more m^{6}A-regulated circRNAs will be developed as biomarkers in body fluids. However, the technical problem of detecting m^{6}A levels of specific circRNAs or measuring the content of circRNAs with low abundance remains to be solved.

In addition, given that circRNAs are usually expressed in a tissue- or cell-type-specific manner, they are considered potentially effective therapeutic targets. Recently, several tools that can effectively target circRNAs have been developed. Synthetic circRNAs that enhance circRNA expression *in vitro* could be delivered to target cells. However, these foreign circRNAs can also induce immune system activation *in vivo*.[106] Strategies that introduce chemical modifications such as m^{6}A modification have been explored to reduce synthetic circRNA immunogenicity.[139] Therefore, m^{6}A-modified circRNAs further broaden their clinical applications.

Accumulating studies have reported that m^{6}A and its regulators serve as new biomarkers for diagnosis and prognosis in various diseases. These core members of m^{6}A methylation usually exert important functions in many biological processes and represent effective therapeutic targets. Recently, m^{6}A inhibitors have been developed and widely applied in clinical treatment, including the FTO inhibitors MO-I-500, R-2-hydroxyglutarate (R-2HG), FB23-2, FTO-04, meclofenamic acid (MA), and the ALKBH5 inhibitor MV1035. However, few inhibitors targeting m^{6}A writers and readers have been reported to date, which may be a research direction for future studies. In addition, circRNAs could affect m^{6}A levels by competitively binding to or directly targeting m^{6}A regulatory proteins. The interactions between m^{6}A and circRNAs provide novel insights into the mechanisms controlling physiological and pathological processes and promote the identification of critical targets for the diagnosis and treatment of diseases.

ACKNOWLEDGMENTS

This work was supported by grants from the National Natural Science Foundation of China (grant nos. 81972806, 82073288, and 81802093), the Nanjing Medical and Health Scientific Research Project (grant no. YKK21137), the Jiangsu Provincial Key Research and Development Plan (grant no. BE2019614), the Elderly Health Research Project of Jiangsu Province (grant no. LR2021017), and the Specialized Cohort Research Project of Nanjing Medical University (NMUC2020035).

AUTHOR CONTRIBUTIONS

T.X., B.H., and H.S. conceived the study, drafted the manuscript, and drew the diagrams. M.X. and J.N. screened the literature and created the tables. S.W. and Y.P. performed the quality assessment, supervised the study, and revised the manuscript. All of the authors approved the final version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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