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REVIEW

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m^{6}A-modified circRNAs: detections, mechanisms, and prospects in cancers

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Abstract

Circular RNAs (circRNAs) have become a research hotspot in recent years with their universality, diversity, stability, conservativeness, and spatiotemporal specificity. N^6-methyladenosine (m^{6}A), the most abundant modification in the eukaryotic cells, is engaged in the pathophysiological processes of various diseases. An increasing amount of evidence has suggested that m^{6}A modification is common in circRNAs and is associated with their biological functions. This review summarizes the effects of m^{6}A modification on circRNAs and their regulation mechanisms in cancers, providing some suggestions of m^{6}A-modified circRNAs in cancer therapy.

Keywords: CircRNA, Non-coding RNAs, N6-methyladenosine, Metabolism, Cancers

Background

Circular RNAs (CircRNAs) were first discovered in the 1970s and were initially used to represent splicing errors before serving as a by-product of splicing (Sanger et al. 1976). Subsequently, a large number of biologically significant circRNAs have merged and come to the attention of scholars. Abnormally expressed circRNAs are commonly linked to various human diseases such as cardiovascular diseases (CVDs), kidney diseases, immunity, and cancers (Gomes et al. 2020; Jan van Zonneveld et al. 2021; Chen et al. 2019a; Shang et al. 2019). Therefore, circRNAs hold great promise for cancer diagnosis and treatment thanks to their universality, diversity, stability, conservativeness, and spatiotemporal specificity (Kristensen et al. 2019).

More than 170 chemically distinct types of modifications have been identified in messenger RNAs (mRNAs) and a few non-coding RNAs (ncRNAs) of eukaryotes, bacteria and archaea, giving rise to RNA epigenetics (Boccaletto et al. 2022). The most popular RNA modifications include N^6-methyladenosines (m^{6}A), 5-methylcytosines (m^{5}C), 5-hydroxymethylcytosine (5hmC), N^1-methyladenosines (m^{1}A), N^6, 2′-Odimethyladenosine (m^{6}Am), 7-methylguanine (m^{7}G), and pseudouridine (Ψ) (Nombela et al. 2021). Among them, the m^{6}A modification is the most abundant base modification in eukaryotic cells with a typical consensus sequence RRACH motif (R = G or A; H = A, C, or U) (Dominissini et al. 2012). Generally, those bases are enriched in the coding sequence (CDS), 3′-untranslated regions (3′-UTRs), and near stop codons of mRNAs (Meyer et al. 2012).

Recently, the m^{6}A modification in the N^6 position of adenosine has been found in circRNAs (Yang et al. 2017). However, the regulatory network between m^{6}A modification and circRNAs remains complex. This review, centered on the roles of m^{6}A modification on circRNAs, summarizes the existing detection methods and databases for m^{6}A-modified circRNAs. The regulatory mechanisms of m^{6}A-modified circRNAs in cancers and their effects on chemoradiotherapy resistance are reviewed to provide a

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comprehensive understanding of cancer diagnosis and treatment.

Biogenesis, characteristics and biological functions of circRNAs

Biogenesis of circRNAs

CircRNAs have proliferated and are primarily generated by the back-splicing of pre-mRNAs. Four biogenesis models of circRNAs have been discovered, including lariat-driven circularization, intron pairing-driven circularization, RNA binding proteins (RBPs)-driven circularization, and intronic lariat (Kristensen et al. 2019). Besides, a small fraction of intron-derived circRNAs can also be generated by pre-tRNA. Briefly, the tRNA splicing nucleic acid endonuclease (TSEN) complex cleaves the intron-containing pre-tRNA at a typical bulge-helix-bulge (BHB) motif and then the resultant intron termini are joined by RtcB ligase to form a stable circRNA (Lu et al. 2015; Schmidt et al. 2019) (Fig. 1A). CircRNAs can thus be divided into four types based on their origins, including: exonic circRNAs (EcircRNAs), exon–intron circRNAs (EIciRNAs), intronic circRNAs (CiRNAs), and others, such as tRNA intronic circular RNAs (TricRNAs) (Schmidt et al. 2019; Zhang et al. 2013) (Fig. 1B).

Characteristics of circRNAs

CircRNAs are found in nearly all mammals (Ji et al. 2019), plants (Wang et al. 2014), parasites (Broadbent et al. 2015), archaea (Danan et al. 2012), and viruses (Nahand et al. 2020). Particularly approximately 9% of expressed genes in human tissues can generate corresponding circRNAs in human heart, and 20% of genes can produce circRNAs in the brain (Aufiero et al. 2018; Rybak-Wolf et al. 2015). Researchers have validated more than 25,000 human fibroblast RNAs with back-splices as circRNAs (Jeck et al. 2013). Furthermore, the same genes can generate various types of circRNAs through alternative circularization (Salzman et al. 2012). Unlike linear RNAs with 5′ and 3′ ends, circRNAs have a covalently closed loop structure generated from primary transcripts by back-splicing (Jeck et al. 2013). CircRNAs are more stable than linear RNAs because the former ones do not have free ends, and therefore are resistant to foreign chemicals or exonuclease interference, and they have a long half-life of more than 48 h (Suzuki et al. 2006; Enuka et al. 2016). In this sense, circRNA can affect cell functions by accumulating in cells with slower division rates. CircRNAs are also highly conserved. One study has shown that approximately 20% of human circRNAs are homologous to mouse circRNAs (Guo et al. 2014). Another study discovered that approximately 20% of porcine splice sites involved in circRNA production are functionally conserved between mice and humans (Venø et al. 2015). Last but not least, circRNAs, which are dynamically expressed in a spatiotemporal manner, especially during mammalian brain development, have varied expression levels during the developmental process and at different regulation levels, making them more likely to be a disease biomarker (Venø et al. 2015; You et al. 2015).

Biological functions of circRNAs

As research advances, circRNAs have received increased attention for their biological functions, as evidenced by the following aspects. (i) Being as microRNA (miRNA) sponges. Many circRNAs have specific binding sites to miRNAs that can reduce the activity of miRNAs while increasing that of miRNA target genes. CircRNAs, as competing endogenous RNAs (ceRNAs) remain the most classical mechanism of tumor regulation (Hansen et al. 2013). (ii) Interacting with RBPs. Some circRNAs contain specific protein binding sites that bind to RBPs and regulate target RNA, thus fostering the linear splicing of the gene and parental gene transcription (Ashwal-Fluss et al. 2014). (iii) Being translated into proteins. Some circRNAs have proven to be translated by the IRES-dependent mechanism, and ribosomes can be recruited by IRES-transacting factors (ITAFs) to initiate translation in the absence of typical translation initiation factors (Jiang et al. 2021; Xia et al. 2019). Besides, m^6A-modified circRNAs can function in cap-independent translation, which will be discussed further below. (iv) Regulating gene transcription. Some researchers claim that some circRNAs in the nucleus can regulate gene transcription and thus perform specific physiological functions. For example, some CiRNAs and EIciRNAs, such as Ci-ankrd52, EIciPAIP2, and EIciEIF3J, are abundant in the nucleus and associated with RNA Pol II to promote transcription of their parental genes (Li et al. 2015). It is worth mentioning that circRNAs can also act as regulators affecting mRNA translation and stability (Wu et al. 2019a; Huang et al. 2020) (Fig. 1C). Therefore, circRNAs have wide range of biological functions that need further exploration.

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Fig. 1 Biogenesis and biological functions of circRNAs. **A** The biogenesis models of circRNAs include lariat-driven circularization, intron pairing-driven circularization, RBP-driven circularization, intronic lariat, and splicing of pre-tRNA. **B** Based on the origin of circRNA, it can be divided into four categories, namely EcircRNA, EIciRNA, CiRNA, and TricRNA. **C** CircRNAs serve four main biological functions, including acting as miRNA sponges, interacting with RBPs, translating into proteins, and regulating gene transcription

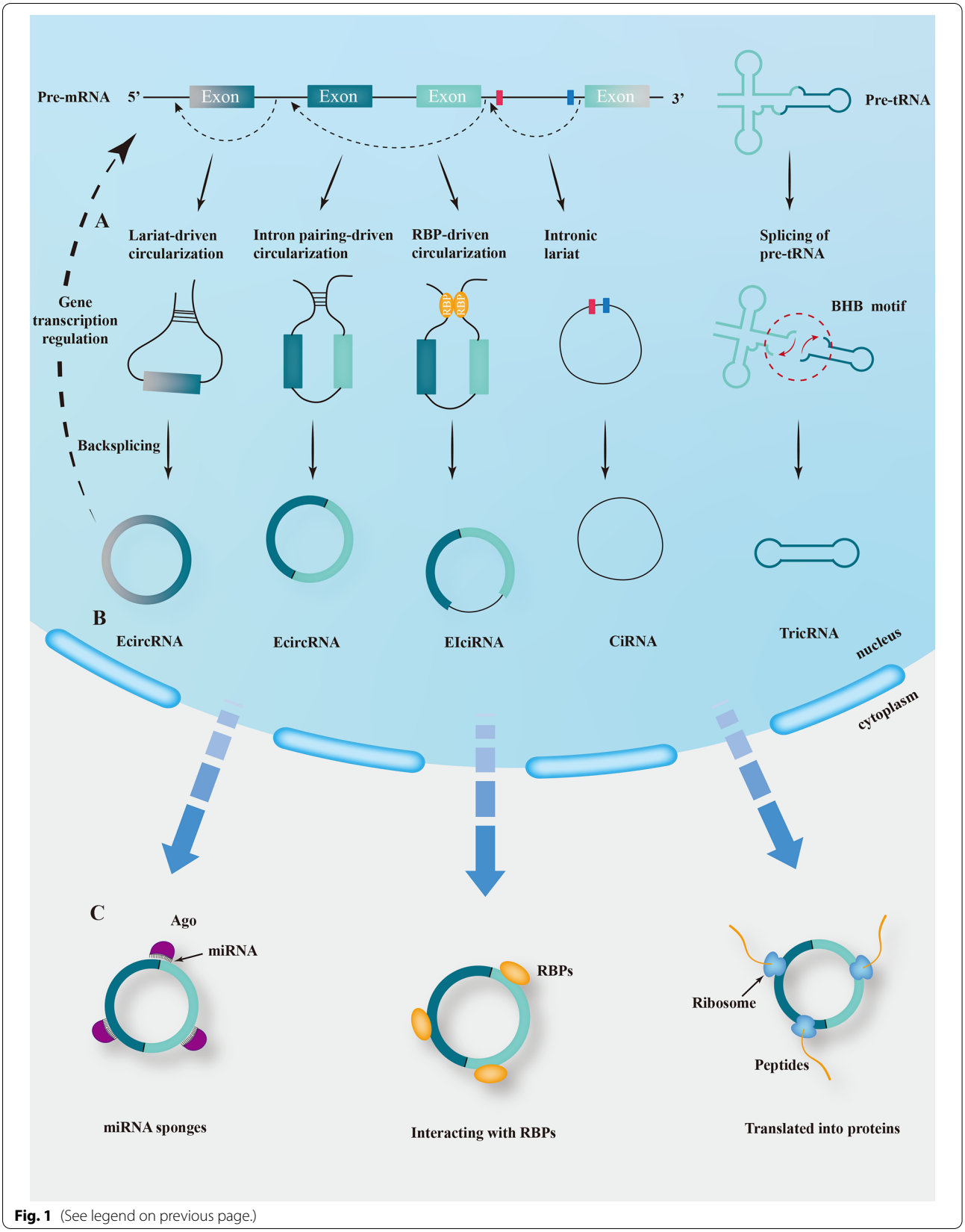

Fig. 1 (See legend on previous page.)

M^{6}A writers, erasers, and readers

M^{6}A modifications on circRNAs can be installed, removed, and recognized by the same m^{6}A regulators in mRNAs, known as "writers" (methyltransferases), "erasers" (demethylases), and "readers" (recognitions).

M^{6}A writers/methyltransferases

Generally, m^{6}A modification are installed by various methyltransferases acting on specific RNAs, but most of them are installed by the multicomponent m^{6}A methyltransferases complex (MTC, also named "writers"), with methyltransferase-like 3 and 14 proteins (METTL3 and METTL14) as its core components (Wang et al. 2016). Other MTC components, such as Wilms Tumor 1 Associated Protein (WTAP) (Ping et al. 2014), Vir-like m^{6}A methyltransferase associated (VIRMA, also called "Virilizer" or "KIAA1429") (Schwartz et al. 2014), RNA recognition motif 15/15B (RBM15/15B) (Patil et al. 2016), Zinc finger CCCH domain-containing protein 13 (ZC3H13) (Knuckles et al. 2018), and Cbl proto-oncogene-like 1 (CBLL1, also known as "HAKAI") (Bawankar et al. 2021), also play roles in facilitating the complex's recruitment to specific sites and maintaining its stability. Aside from the enzymes mentioned above involved in MTC formation, methyltransferase-like 16 (METTL16) (Pendleton et al. 2017), methyltransferase-like 5 (METTL5) (Tran et al. 2019), and Zinc finger CCCH-Type containing 4 (ZCCHC4) (Ma et al. 2019) have been discovered to be independent RNA methyltransferases. However, these methyltransferases can only catalyze a few m^{6}A residues in RNAs (Pendleton et al. 2017; Tran et al. 2019; Pinto et al. 2020).

M^{6}A erasers/demethylases

M^{6}A methylation is a dynamic, multi-layered, and reversible process that can be removed by erasers (also known as "demethylases"). Fat mass and obesity-associated protein (FTO, also known as "ALKBH9") and AlkB homolog 5 (ALKBH5) belong to the AlkB subfamily of Fe (II)/α-ketoglutaric acid (αKG) dioxygenase, and they can catalyze the demethylation of m^{6}A in both αKG and Fe (II) dependence(Jia et al. 2011; Zheng et al. 2013).

M^{6}A readers/recognitions

Numerous studies have revealed that m^{6}A modifications can be recognized by various binding proteins (also called readers) to perform specific biological functions. To date, several readers have been extensively studied. Take YT521-B homology (YTH) domain family for example. It contains five proteins: YTH domain family protein 1 (YTHDF1), YTH domain family protein 2 (YTHDF2), YTH domain family protein 3 (YTHDF3), YTH domain containing 1 (YTHDC1), and YTH domain containing 2 (YTHDC2) (Liu et al. 2015). The first three are typically found in the cytoplasm to perform their functions. Among them, YTHDF2 can interact with the carbon catabolite repressor 4-negative on TATA (CCR4-NOT) complex to transport RNA to the processing body (P-body), thereby degrading RNA (Du et al. 2016). Besides, YTHDF1 and YTHDF3 have been found to act synergically to mediate m^{6}A modifications in RNAs and affect the initiate translation of RNA with eukaryotic initiation factor 3, 4E, and 4G (eIF3, eIF4E, and eIF4G), poly(A) binding protein (PABP), and the 40S ribosomal subunit in a cap-dependent manner (Wang et al. 2015; Shi et al. 2017). However, a recent study has found that YTHDF2 can also exist in the nucleus, interact with m^{6}A modifications on RNA within R-loops, and destabilize the RNA: DNA hybrids, thus regulating the accumulation of R-loops, and playing a role in safeguarding genomic stability (Abakir et al. 2020). YTHDC1 is also nuclear enriched and primarily involved in the selective splicing and nuclear transport of m^{6}A transcripts (Widagdo et al. 2022). YTHDC2, which occurs in the cytoplasm and plays a vital role in RNA decay via interactions with adaptor proteins, and in RNA translation efficiency (Wojtas et al. 2017; Mao et al. 2019). In addition to the YTH domain family, heterogeneous nuclear ribonucleoprotein C1/C2 (HNRNPC), heterogeneous nuclear ribonucleoprotein G (HNRNPG), and heterogeneous nuclear ribonucleoprotein A2B1 (HNRNPA2B1), as part of the heterogeneous nuclear ribonucleoprotein (HNRNP) family are involved in alternative splicing and nuclear RNA processing (Alarcón et al. 2015; Liu et al. 2017). Furthermore, it has been proposed that eIF3 initiates translation in a cap-independent manner by binding to the m^{6}A sites in the 5′-UTR of mRNAs (Meyer et al. 2015), while insulin-like growth factor 2 mRNA-binding protein 1/2/3 (IGF2BP1/2/3) can enhance the stability and translation of the target RNAs in the cytoplasm (Zhang et al. 2018; Wu et al. 2019b). Similar to IGF2BP1/2/3, fragile X mental retardation protein (FMRP) and proline-rich spiral coil 2A (PRRC2A) can also maintain the stability of their target RNAs. Furthermore, it is worth noting that FMRP can also occur in the nucleus and take part in the nuclear export of m^{6}A-enriched RNAs (Hsu et al. 2019) (Fig. 2).

In summary, those m^{6}A regulators, particularly readers, are complex and diverse. Their effects on m^{6}A-modified circRNAs in cancers are discussed in detail below.

Detection methods and databases for m^{6}A-modified circRNAs

Over the past decades, further research into the functions of m^{6}A-mediated circRNAs has been limited by a lack of suitable detection methods and databases.

However, with the continuous improvement of multiple detection methods and databases, especially the emergence of the next-generation sequencing (NGS), the field of m^{6}A methylation has seen a dramatic shift.

Quantitative and semi-quantitative detection of m^{6}A-modified circRNAs

As a semi-quantitative method for determining the level of overall m^{6}A-modified circRNAs, dot blot is easy to operate and time-saving, but it only can confirm the presence of m^{6}A or compare the amount of m^{6}A in different groups, but cannot quantify or locate m^{6}A (Zhou et al. 2017). In addition, the m^{6}A level detection is a colorimetric method for quantifying the overall level of m^{6}A RNA methylation in total RNAs, mRNAs, and ncRNAs. The concept of the test is similar to enzyme-linked immunosorbent assay (ELISA) and easy to operate (Ge et al. 2020). However, Ribonuclease R (RNase R) should be first used to de-linearize for quantifying the overall m^{6}A level in total circRNAs and more research papers will be required to validate the method in the future. Besides, Methylated RNA immunoprecipitation (MeRIP) assay (m^{6}A RIP), is a method for enriching m^{6}A-modified circRNAs by using an anti-m^{6}A antibody and quantitative real-time polymerase chain reaction (qPCR) to identify the enriched circRNAs. This method is convenient and only requires a kit to perform an experiment, but it lacks specificity (Chen et al. 2021). Moreover, the m^{6}A-circRNA epitranscriptomic microarray in combination with a dual-color fluorescence microarray labeling system and RNA modification immunoprecipitation, allows for the quantitative detection of the percentage of epigenetic modifications in each transcript with a low total RNA requirement and high specificity. This method, however, is not widely used and deserves more attention (Fan et al. 2022) (Table 1).

The detection of m^{6}A modification sites in circRNAs

Although most relevant methods focus on detecting m^{6}A modifications in linear RNAs, the precise detection of m^{6}A modification sites in circRNAs remains uncommon. Methylated RNA immunoprecipitation and sequencing (MeRIP-seq/m^{6}A-seq), is a predominant method for detecting m^{6}A modifications in RNAs. It mainly combines anti-m^{6}A antibody with m^{6}A-containing RNA fragments for NGS. The m^{6}A-seq approach has some limitations: (i) It can only identify m^{6}A hypermethylation enrichment regions on RNAs with a resolution of about 100nt, but cannot locate individual m^{6}A sites; (ii) It requires a large number of total RNA samples due to its low sensitivity; (iii) Antibodies to m^{6}A can recognize modifications similar to m^{6}A, such as m^{6}Am, with less specificity (Dominissini et al. 2012; Antanaviciute et al. 2017). Notably, a variety of antibody-independent methods for detecting m^{6}A modifications have been discovered in recent years. For example, MazF PCR is a single-base m^{6}A detection method that uses the m^{6}A-sensitive RNA endonuclease MazF, which has been found to cleave RNAs with non-methylated ACA sequence, but not those with the methylated m6ACA sequence. However, to cover all the RRACH motifs in the transcriptome, new enzymes that recognize more universal sequence motifs must be explored (Imanishi et al. 2017). Besides, the T3 DNA ligase-dependent PCR assay is a highly sensitive and selective single-base detection that can locate m^{6}A modification fraction at any specific RNA site. It is worth noting that both MazF PCR and ligase-dependent PCR assays for detecting m^{6}A sites in circRNAs require RNase R to digest linear RNA before performing such validations (Liu et al. 2018). Furthermore, nanopore-based direct RNA sequencing (nanopore DRS) is another single-base detection method that locate m^{6}A modifications in circRNAs by enriching circRNAs in samples, fragmenting and sequencing them on nanopore platforms, with high efficiency and simplicity (Wang et al. 2020). However, more studies are needed to validate the application of the aforementioned antibody-independent methods in m^{6}A-related fundamental studies and clinical diagnosis (Table 1).

Databases for predicting m^{6}A-modified circRNAs

The databases for predicting m^{6}A methylation sites of circRNAs include Ensembl (Howe et al. 2021), Circm6A (Ye et al. 2021), TransCirc (Huang et al. 2021), SRAMP (Zhou et al. 2016), RMVar (Luo et al. 2021), RMBase V2.0 (Xuan et al. 2018), circBank (Liu et al. 2019), and DeepM6ASeq (Zhang and Hamada 2018). These databases can predict not only m^{6}A modifications but also circRNAs with miRNA binding sites, protein-coding potential, conservations, mutations, etc. Notably, m6A2Target is a

(See figure on next page.)

Fig. 2 The dynamic and reversible process of m^{6}A modification. The m^{6}A modification can be installed by the multicomponent m^{6}A methyltransferases complex (writers) which includes METTL3, METTL14, WTAP, VIRMA, RBM15/15B, ZC3H13 and CBLL1, as well as independent RNA methyltransferases such as METTL16, METTL5, ZCCHC4, and removed by demethylases (erasers) FTO and ALKBH5. Various binding proteins (readers) can then recognize the m^{6}A modification to perform specific biological functions. In the nucleus, m^{6}A can be identified by YTHDC1, HNRNPC/G, HNRNPA2B1, YTHDF2 and FMRP, and is involved in RNA alternative splicing, nuclear RNA processing, R-loop degradation, and RNA export. In the cytoplasm, m^{6}A can be identified by YTHDF1/2/3, YTHDC2, IGF2BPs, eIF3, FMRP and PRRC2A, and regulates RNA stability, translation, and degradation

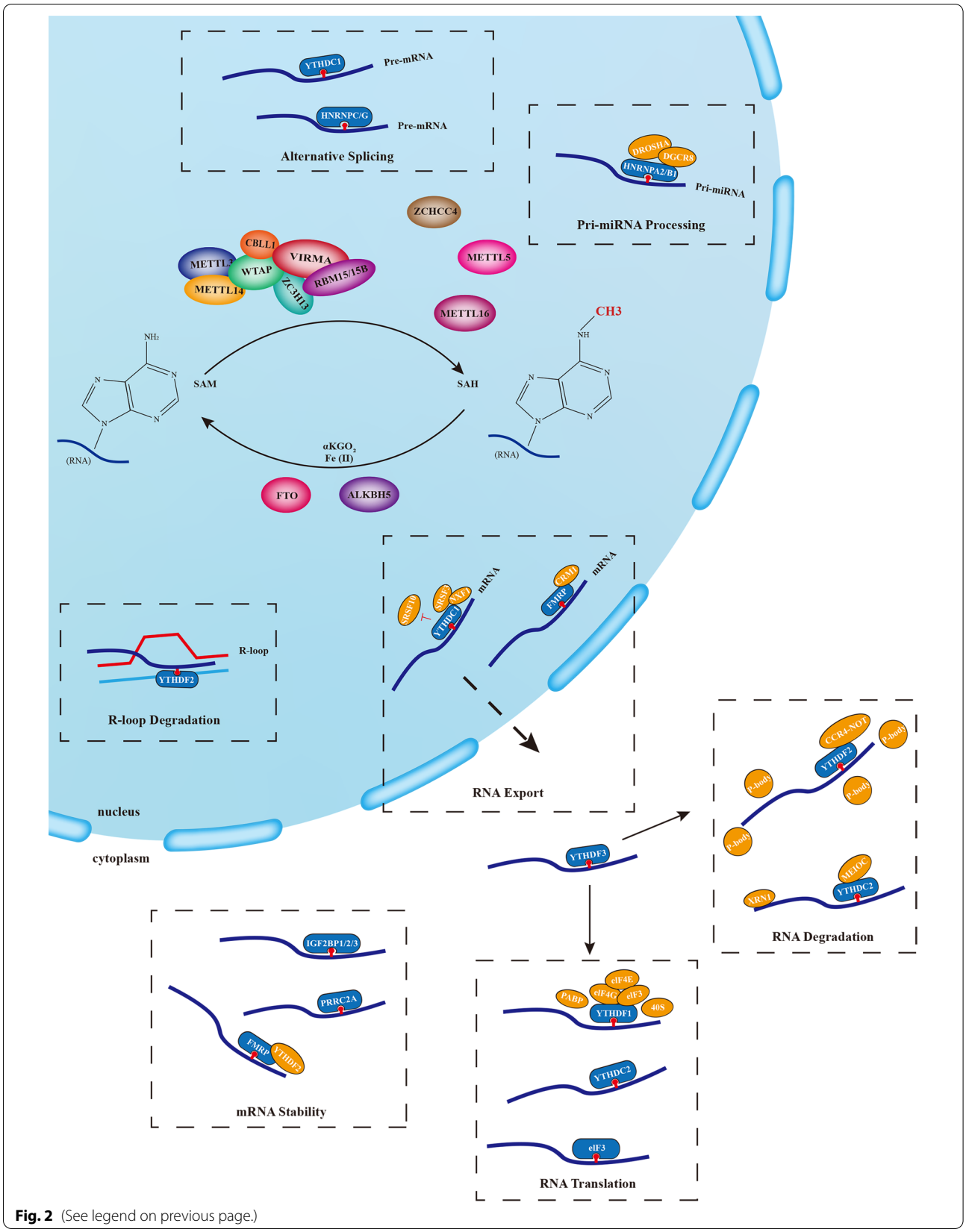

Fig. 2 (See legend on previous page.)

Table 1 Detection methods for m^{6}A-modified circRNAs

Methods	References
Quantitative and semi-quantitative detection	
Dot blot	Zhou et al. (2017)
M^{6}A level detection	Ge et al. (2020)
MeRIP assay/m^{6}A RIP	Chen et al. (2021)
M^{6}A-circRNA epitranscriptomic microarray	Fan et al. (2022)
The detection of m^{6}A modification sites	
MeRIP-seq/m^{6}A-seq	Dominissini et al. (2012), Antanaviciute et al. (2017)
MazF PCR	Imanishi et al. (2017)
T3 DNA ligase-dependent PCR	Liu et al. (2018)
Nanopore DRS	Zhao et al. (2019)

novel comprehensive database for exploring the target genes of writers, erasers, and readers of m^{6}A modification (Deng et al. 2021). Thanks to their convenience, simplicity, and data visualization, those databases facilitate scientific research (Table 2).

Role of m^{6}A modifications on circRNAs

M^{6}A modification mediates circRNAs translation

Accumulating evidence indicates that circRNAs code mainly through the IRES-driven translation and m^{6}A-driven translation. Studies found that circRNAs containing m^{6}A residues can be translated cap-independently. For example, Yang et al. discovered that the m^{6}A-driven translation of circRNAs relies on the reading protein YTHDF3, as well as eukaryotic translation initiation factor 4 gamma 2 (eIF4G2) and eukaryotic initiation factor 3A (eIF3A) and that this process can be enhanced by methyltransferase METTL3/14 and inhibited by demethylase FTO. Moreover, further assays have indicated that an m^{6}A site is sufficient to initiate translation and identify 33 peptides encoded by the back-splice junctions of m^{6}A-modified circRNAs. These 33 peptides do not match any known proteins in the UniProt database but can be identified through proteomic analyses, suggesting that the m^{6}A-driven translation of circRNAs widespread in the human transcriptome (Yang et al. 2017). Similarly, in human papillomavirus (HPV), circE7 with m^{6}A modification can be translated into the E7 tumor protein (Zhao et al. 2019). Besides, studies have pointed out that m^{6}A modifications can initiate and regulate circRNAs translation. Previous studies have discovered that circ-ZNF609 can be translated through the IRES-driven manner, while the latest one has identified that m^{6}A-modified circ-ZNF609 can drive cap-independent translation through YTHDF3 and elF4G2. The above-mentioned findings suggest that the possibility of an interaction between the two forms that drive the translation of circRNAs. However, the specific correlation between them needs to be further explored (Legnini et al. 2017; Timoteo et al. 2020) (Fig. 3A).

To summarize, all those findings provide more possibilities for exploring the translation of m^{6}A-driven circRNAs.

Table 2 Databases for predicting m^{6}A-modified circRNAs

Name	Website	Characteristics	Reference
Ensembl	http://rapid.ensembl.org	It is a genome browser can be used to identify m^{6}A modification sites with the RRACH motif	Howe et al. (2021)
Circm6A	https://github.com/canceromics/circm6a	It is a powerful tool for detecting m^{6}A modification of circRNA	Ye et al. (2021)
TransCirc	https://www.biosino.org/transcirc/	It is a database that mainly predict translatable circRNA and circRNA m^{6}A modification sites	Huang et al. (2021)
SRAMP	http://www.cuilab.cn/sramp	It can extract and integrate the sequence and predict structural features around m^{6}A sites	Zhou et al. (2016)
RMVar	http://m6avar.renlab.org	It can be used to search for m^{6}A-associated variants and diseases	Luo et al. (2021)
RMBase V2.0	http://rna.sysu.edu.cn/rmbase	It is a comprehensive database for exploring post-transcriptionally modifications of RNAs and their relationships with microRNA binding events, disease-related SNPs, and RBPs	Xuan et al. (2018)
circBank	www.circbank.cn	It is a comprehensive database for predicting circRNAs with miRNA binding sites, protein coding potential, conservations, mutations, and m^{6}A modifications	Liu et al. 2019)
DeepM6ASeq	https://github.com/rreybeyb/DeepM6ASeq	It is a deep-learning-based framework to predict m^{6}A-containing sequences and visualize saliency map for sequences	Zhang and Hamada (2018)
m6A2Target	http://m6a2target.canceromics.org/#/home	It is a comprehensive database for the target gene of writers, erasers and readers of m^{6}A modification	Deng et al. (2021)

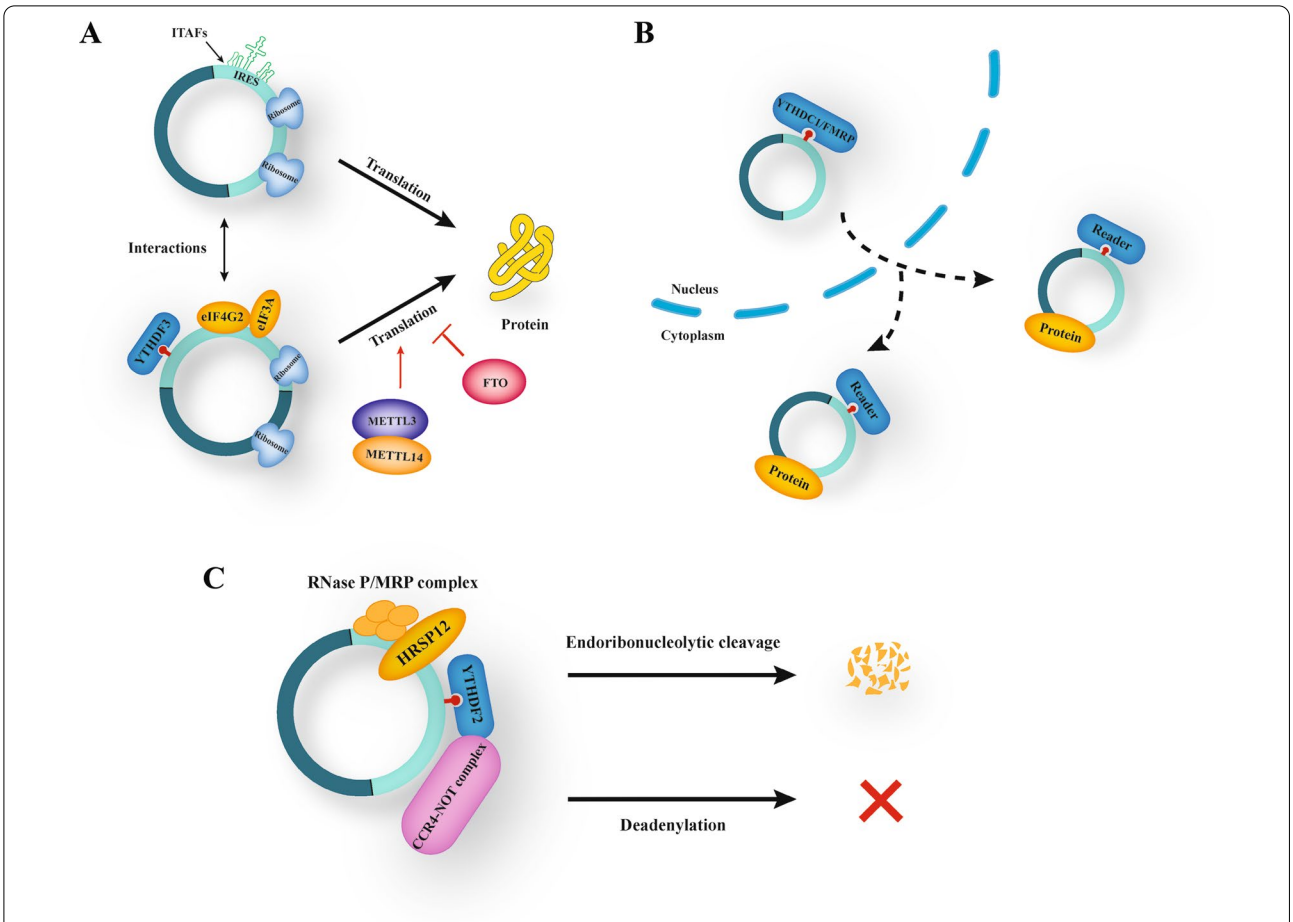

Fig. 3 Role of M^{6}A modifications on circRNAs. **A** M^{6}A modification mediates circRNAs translation. M^{6}A-driven translation of circRNAs relies on YTHDF3, eIF4G2 and eIF3A. Meanwhile, the process can be enhanced by METTL3/14 and inhibited by FTO. Besides, it is suggested that an interaction may exist between the IRES-driven translation and m^{6}A-driven translation. **B** M^{6}A modification mediates circRNAs nucleoplasmic transport. The m^{6}A readers, such as YTHDC1 and FMRP, could induce the nuclear and cytoplasmic transport of circRNAs. **C** M^{6}A modification regulates the stability of circRNAs. M^{6}A-modified circRNAs can be endoribonuclease-cleaved via YTHDF2-HRSP12-RNase P/MRP axis

M^{6}A modification mediates nucleoplasmic transport of circRNAs

In recent years, many published articles have shown that individual circRNAs can be transported into the cytoplasm during biogenesis and development, competing with other RNAs for binding by RBPs or miRNAs (Memczak et al. 2013). Therefore, it is crucial to understand how circRNAs export from nucleus to the cytoplasm. In drosophila, researchers have found that the Drosophila DExH/D-box helicase at 25E (Hel25E) interference significantly enriches circRNAs in the nucleus. In human cells, circRNAs have been discovered to be transported from the nucleus to the cytoplasm in a transcript-length-dependent manner via drosophila Hel25E and its human homologs, ATP-dependent RNA helicase DDX39A (also termed as nuclear RNA helicase URH49) and spliceosomal RNA helicase DDX39B (also termed as dead box protein UAP56) (Huang et al. 2018). Besides, Chen et al. identified that circ1662 overexpression increases the nuclear yes-associated protein 1 (YAP1) and decreases the cytoplasmic YAP1, indicating that circ1662 could promote YAP1 nuclear transport. Further function assays have confirmed that circ1662 promotes colorectal cancer (CRC) invasion and migration by accelerating YAP1 nuclear transport (Chen et al. 2021). In addition, the m^{6}A reader YTHDC1 can bind to circNSUN2 and facilitate circNSUN2 to export from the nucleus to the cytoplasm in an m^{6}A-dependent manner, and to promote colorectal liver metastasis through the circNSUN2-IGF2BP2-High Mobility Group AT-Hook 2 (HMGA2) RNA–protein ternary complex in the cytoplasm (Chen et al. 2019b). Furthermore, YTHDC1 and FMRP have been identified as readers to recognize HBV transcripts with m^{6}A methylation modification and facilitate their transport to the cytoplasm (Kim et al. 2021) (Fig. 3B).

Consequently, the m^{6}A modification can affect the nuclear and cytoplasmic transport of circRNAs by interacting with proteins.

M^{6}A modification regulates the stability of circRNAs

RNase R and actinomycin D assays have shown that circRNAs are more stable than their origin genes, because they are not easily degraded by nucleic acid exonucleases and have a long half-life. Nonetheless, a recent study has pointed out that circRNAs can be degraded in some unique manners. For example, Hansen et al. revealed that the removal of the circular cerebellar degeneration associated protein 1 (CDR1) antisense transcripts with perfect complementary miRNA target sites could be mediated by miR-671 in an Argonaute2 (Ago2)-slicer-dependent manner. However, it does not work for circRNAs that lack miRNA sponge function or specific miRNA target sites (Hansen et al. 2011). Another study has reported that the depletion of GW182, a key component of P-body and RNA interference (RNAi) machine, can accumulate steady-state circRNA transcripts. However, that of other P-body components or RNAi machine factors does not affect circRNA levels, indicating that GW182 is a major factor in circRNA degradation. Nevertheless, the specific mechanisms remain to be further investigated (Jia et al. 2019). Aside from the above-mentioned findings, YTHDF2-heat-responsive protein 12 (HRSP12)-ribonuclease P (RNase P)/mitochondrial RNA processing (MRP) is the most common way of endoribonucleolytic cleavage of m^{6}A-modified circRNAs. HRSP12 acts as an adapter protein that links YTHDF2 and RNase P/MRP, rapidly degrading YTHDF2-bound circRNAs (Park et al. 2019) (Fig. 3C).

Therefore, the complexities in the degradation of m^{6}A circRNAs could contribute to the more dynamic regulation of m^{6}A-modified circRNAs during various biological and physiological processes.

M^{6}A-modified circRNAs in cancers

Colorectal cancer

Colorectal cancer (CRC) has been reported to rank third in incidence, and second in mortality according to the latest research. It accounts for about one in ten cancer cases and deaths (Sung et al. 2021). Therefore, specific mechanisms must be explored to better understand the CRC progression.

By using the MeRIP assay, Gene Expression Omnibus (GEO), and The Cancer Genome Atlas (TCGA) databases, researchers have found that circ3823 is enriched in the m^{6}A precipitated fraction and have speculated that YTHDF3 and ALKBH5 cooperate with YTHDF2 to degrade circ3823, demonstrating that circ3823 might promote CRC growth, metastasis, and angiogenesis via circ3823/miR-30c-5p/Transcription factor 7 (TCF7) axis (Guo et al. 2021). Besides, refractory metastatic CRC is usually the leading cause of death in CRC patients (Hofheinz and Stintzing 2019). For example, Chen et al. demonstrated that METTL3 can induce circ1662 formation by installing m^{6}A modifications in its flanking reverse complementary sequences via MeRIP assay, thus promoting epithelial-mesenchymal transition (EMT) and accelerating lung metastases of CRC via the YAP1-mothers against decapentaplegic homolog 3 (SMAD3) axis (Chen et al. 2021). Additionally, another study has identified that m^{6}A-modified circNSUN2 is frequently upregulated in CRC patients with liver metastasis (LM), indicating a lower patient survival. MeRIP assay and other assays first verified that circNSUN2 is highly enriched in the m^{6}A precipitated fraction and YTHDC1 can promote cytoplasmic export of m^{6}A-modified circNSUN2. Further assays have indicated that circNSUN2 enhances the stability of HMGA2 mRNA by forming a circNSUN2/IGF2BP2/HMGA2 ternary complex in the cytoplasm, thus leading to the LM of CRC (Chen et al. 2019b).

In conclusion, those findings suggest that m^{6}A-modified circRNAs may play a vital role in the CRC progression and serve as a potential diagnostic and therapeutic target for CRC, especially the metastasis-related CRC.

Gastric cancer

In the latest global cancer report, gastric cancer (GC) is the fifth most common cancer and the fourth leading cause of cancer death worldwide (Sung et al. 2021). Therefore, further study of the molecular mechanism underlying GC is required.

Zhang et al. first predicted the potential m^{6}A sites of the top 20 differentiated expressed circRNAs (DECs) by adopting the SRAMP database and m^{6}A RIP assays, indicating that the m^{6}A level of DECs is positively correlated with the DEC expression level in gastric tissues and may be closely related to circRNA functionality. Nevertheless, more research into the potential functions and mechanisms of m^{6}A modification on identified DECs in poorly differentiated gastric adenocarcinoma (PDGA) is needed (Zhang et al. 2020). M^{6}A-circRNA epitranscriptomic microarray and MeRIP assays have revealed that METTL14 can regulate the m^{6}A level and expression of circORC5, and that METTL14-mediated circORC5 can sponge miR-30c-2-3p to regulate AKT1 substrate 1 (AKT1S1) and eukaryotic translation initiation factor 4B (EIF4B) expression in GC cells, thereby promoting GC progression (Fan et al. 2022).

Overall, those findings shed light on how m^{6}A-modified circRNAs contribute to GC.

Liver cancer

Liver cancer is the sixth most common cancer and the third leading cause of cancer death worldwide, among which hepatocellular carcinoma (HCC) comprises 75–85% (Sung et al. 2021). Several studies have shown that m^{6}A-modified circRNAs are involved in HCC regulation.

In the study of Chi et al., circMAP2K4 was validated to promote HCC biogenesis via the miR-139-5p/YTHDF1 axis. Then, the expression and prognostic value of all m^{6}A RNA methylation modulators and the biological pathways were evaluated by TCGA and International Cancer Genome Consortium (ICGC) databases, indicating that the circRNA regulatory network based on hsa-miR-139-5p/YTHDF1 axis is involved in regulating m^{6}A RNA methylation modulators (Chi et al. 2021). Besides, Liu et al. observed that KIAA1429 is negatively correlated with m^{6}A-modified circDLC1 after the intersection of RNA-seq and m^{6}A-seq approaches. Further assays have found that circDLC1 binds to Human Antigen R (HuR) and blocks the interaction between HuR and matrix metalloproteinase 1 (MMP1) mRNAs, suggesting that m^{6}A-regulated circDLC1 may serve as a therapeutic target for HCC (Liu et al. 2021). Additionally, MeRIP-seq, SRAMP database, and m^{6}A RIP assays have confirmed that circHPS5 is highly m^{6}A-modified, and METTL3 can mediate the circHPS5 formation. YTHDC1 can expedite the cytoplasmic output of m^{6}A-modified circHPS5, making circHPS5 act as a miR-370 sponge to regulate HMGA2 expression and accelerate HCC cell development (Rong et al. 2021).

Hence, those findings convincingly indicated that m^{6}A regulated-circRNAs may serve as potential therapeutic targets for liver cancer.

Breast cancer

Breast cancer (BC) is the fifth leading cause of cancer mortality, surpassing lung cancer as the leading cause worldwide (Sung et al. 2021). Therefore, identifying novel mechanisms and therapeutic targets is crucial for BC treatment.

Fortunately, the circBank database and m^{6}A RIP assays have revealed that circMETTL3 is highly enriched in m^{6}A precipitated fraction, and its expression is affected by the m^{6}A modification. CircMETTL3 can sponge miR-31-5p to upregulate cyclin-dependent kinases (CKD1) expression, thus promoting BC progression (Li et al. 2021a).

Those findings indicated that circMETTL3 may act as a potential therapeutic target for BC. Nevertheless, the role of m^{6}A-modified circRNAs in BC is rarely reported and deserves more attention.

Cervical cancer

Cervical cancer (CC) is the fourth most commonly diagnosed cancer and the fourth leading cause of cancer death in women (Sung et al. 2021).

M^{6}A-RIP assay has confirmed that METTL3 can mediate the m^{6}A modification level of human papillomavirus (HPV)-derived circE7. Further assays have revealed that circE7 can encode E7 oncoprotein in a heat-shock regulated manner and that the mutation of the potential m^{6}A motifs of circE7 can strongly inhibit E7 oncoprotein expression, implying that m^{6}A-modified circE7 plays a vital role in the translation mechanism (Zhao et al. 2019). Besides, another study has found that m^{6}A-modified circARHGAP12 can interact with the m^{6}A reader IGF2BP2 to enhance forkhead box M1 (FOXM1) mRNA stability and thus allow CC cells to proliferate and migrate(Ji et al. 2021).

In summary, those achievements might provide ideas for the targeted therapy based on the mechanisms of m^{6}A-modified circRNAs regulating CC tumorigenesis.

Lung cancer

Lung cancer remains the leading cause of cancer morbidity and mortality worldwide, with non-small cell lung cancer (NSCLC) accounting for about 80–85% (Sung et al. 2021). Despite recent advances in NSCLC treatment, the overall cure and survival rates remain low (Hirsch et al. 2017). Therefore, it is crucial to study and figure out the molecular mechanism of NSCLC to improve its prognosis.

In the study by Li et al., the MeRIP assay revealed that circNDUFB2 is considerably enriched in m^{6}A modification, and that METTL3/14 plays a significant role in affecting the interactions between circNDUFB2 and IGF2BPs. CircNDUFB2 not only acts as a scaffold by forming a tripartite motif containing 25 (TRIM25)/circNDUFB2/IGF2BPs ternary complex to facilitate the degradation of IGF2BPs, but it also triggers cellular immune responses by activating retinoic acid-inducible gene-I (RIG-I), thereby regulating NSCLC progression (Li et al. 2021b).

To sum up, their study broadens the knowledge of m^{6}A-modified circRNAs action in NSCLC progression, implying that circNDUFB2 may have immunotherapy potentials for NSCLC.

Glioma

Glioma, an intracranial malignant tumor, has a high mortality and morbidity rate (Ostrom et al. 2014). Recent research into the molecular mechanism of glioma malignant proliferation has sparked widespread concern.

By using m^{6}A level detection and MeRIP assays, Wu et al. discovered that METTL3-mediated m^{6}A

modification can enhance the stability and expression of circDLC1, thereby promoting the competitive binding of circDLC1 and miR-671-5p, facilitating Catenin Beta Interacting Protein 1 (CTNNBIP1) transcription, and ultimately suppressing the malignant proliferation of glioma cells (Wu et al. 2022).

This study first reported the mechanism of METTL3-mediated m^{6}A modification of circDLC1 on the malignant proliferation of glioma cells, shedding light on glioma treatment.

M^{6}A-modified circRNAs and tumor chemoradiotherapy resistance

Increasing evidence suggests that m^{6}A-modified circRNAs may also contribute to cancer chemotherapy resistance. For example, in sorafenib-resistant HCC cells, Xu et al. demonstrated that the m^{6}A modification can increase its stability to regulate circRNA-SORE expression by using SRAMP, RMBase v2.0 database, and MeRIP assays, and that increased circRNA-SORE can sponge miR-103a-2-5p and miR-660-3p to activate Wingless-types/beta-catenin (Wnt/β-catenin) pathway and induce sorafenib resistance (Xu et al. 2020). Besides, the SRAMP database and MeRIP assays discovered that circMAP3K4 is highly enriched in the m^{6}A modification, and further investigations revealed that IGF2BP1-mediated m^{6}A recognition can translate circMAP3K4 into circMAP3K4 translation produced a 455 amino acid protein (circMAP3K4-455aa), thus preventing HCC cells from cisplatin-induced death (Duan et al. 2022). Additionally, recent research has explored how radiotherapy affects hypopharyngeal squamous cell carcinoma (HPSCC) prognosis. Diagnostics and treatments based on molecular biology are urgently needed to mitigate toxicity and adverse effects. For example, one study using MeRIP assays confirmed that METTL3 could stabilize the expression of circCUX1 through m^{6}A modification in head and neck tumor cell lines. Notably, circCUX1 can bind to caspase 1 mRNA and inhibit its expression, thereby inhibiting caspase 1 mediated inflammation and developing tolerance to radiotherapy (Wu et al. 2021) (Table 3).

To sum up, those findings suggest that m^{6}A-modified circRNAs may act as a potential therapeutic target for tumor chemotherapy and radiotherapy tolerance.

Conclusion and remarks

Much evidence supports that epigenetic modification can affect RNAs involved in cellular processes. The m^{6}A modification on circRNAs has been gradually identified and is also critical for human development and disease progression. Similar to the modification in mRNAs, the m^{6}A modification in circRNAs can be written, removed, and read by the same regulators and perform specific biological functions. In terms of the biological function, m^{6}A modification can regulate circRNA translation, nuclear-cytoplasmic transport, and degradation. Most importantly, m^{6}A-modified circRNAs can participate in various physiological and pathological processes, particularly in cancers. That means m^{6}A-modified circRNAs have a wide range of biological functions and a broad research space in the future.

Previous studies have shown that circRNAs are stable in blood and body fluids due to their unique structure of single-stranded, covalently closed circular transcripts, which can help them avoid exonuclease degradation. Hence, abnormal-expressed circRNAs in peripheral blood or body fluids have been proven useful as biomarkers for tumor diagnosis (Ge et al. 2022). One recent study has found that the m^{6}A level in peripheral blood RNA combined with current tumor markers such as carcinoembryonic antigen (CEA) or m^{6}A demethylases ALKBH5 and FTO can improve the diagnostic value of m^{6}A, revealing that the m^{6}A level in peripheral blood RNA can be a potential biomarker for GC diagnosis and follow-up (Ge et al. 2020). Additionally, several cancer treatments, including surgery, chemotherapy, radiotherapy, targeted therapy, and immunotherapy, have been widely applied over the past few decades, generally prolonging disease-free survival (PFS) and overall survival (OS) rates among cancer patients (Maji et al. 2018; Esfahani et al. 2020). However, due to the enormous tumor heterogeneity, cancer cells typically show primary or acquired drug resistance, leading to cancer treatment failure. For this reason, an increasing amount of research is focusing on less toxic therapies based on molecular biology. Aside from the m^{6}A-modified circRNAs as therapeutic targets for tumor chemotherapy and radiotherapy resistance, the m^{6}A regulators have also become therapeutic targets for tumors. For example, one research has revealed that ALKBH5-mediated alterations in m^{6}A density can regulate the splicing and expression of mRNAs with potential roles in controlling tumor growth, thus suggesting that ALKBH5, the m^{6}A demethylase, can be a potential therapeutic target for cancer treatment alone or in combination with immune checkpoint blockade (ICB) (Li et al. 2020). Nevertheless, more research is needed to comprehensively understanding how m^{6}A regulatory factors function in cancer therapy. Furthermore, some methods for detecting m^{6}A-modified circRNAs, such as dot blot, MeRIP assay, and MeRIP-seq, are widely used. Other methods, such as m^{6}A-circRNA epitranscriptomic microarray, MazF PCR, and nanopore DRS, will require more proof-of-concept studies in the future.

Briefly, more studies on the biological functions and mechanisms of m^{6}A-modified circRNAs are needed,

Table 3 Roles of m^{6}A-modified circRNAs in various cancers

Cancer	CircRNA name	Regulation	M^{6}A component	Fuction	Role in cancer	M^{6}A identification methods and databases	Main mechanisms	References
CRC	circ3823	Up	ALKBH5/YTHDF2/YTHDF3	Eraser/reader/reader	Anti-oncogene	MeRIP assay, GEO and TCGA databases	Sponge miR-30c-5p to upregulate TCF7 expression	Guo et al. (2021)
CRC	circ1662	Up	METTL3	Writer	Oncogene	MeRIP assay	Bind to YAP1 protein and promote its nuclear transport to regulate SMAD3	Chen et al. (2021)
CRC	circNSUN2	Up	YTHDC1/IGF2BP2	Reader/reader	Oncogene	MeRIP assay	Bind to YTHDC1 and promote its export to the cytoplasm, as well as stabilize HMGA2 mRNA via circNSUN2-IGF2BP2-HMGA2 axis	Chen et al. (2019b)
PDGA	A series of circRNAs (circ0077837)	Up/down (down)	–	–	–	SRAMP, m^{6}A RIP	-	Zhang et al. (2020)
GC	circORC5	Up	METTL14	Writer	Anti-oncogene	m^{6}A-circRNA epitranscriptomic microarray, MeRIP assay	Sponge miR-30c-2-3p to regulate AKT1S1 and EIF4B expression	Fan et al. (2022)
HCC	circMAP2K4	Up	YTHDF1	Reader	Oncogene	TCGA and ICGC databases	Sponge hsa-miR-139-5p to regulate the expression of YTHDF1	Chi et al. 2021)
HCC	circDLC1	Down	KIAA1429	Writer	Oncogene	m^{6}A-seq	Bind to HuR protein and block the interaction between HuR and MMP1 mRNAs	Liu et al. (2021)
HCC	circHPS5	Up	METTL3/YTHDC1	Writer/reader	Oncogene	MeRIP-seq, SRAMP, m^{6}A RIP	Sponge miR-370 to regulate HMGA2 expression and expedite its cytoplasmic output	Rong et al. (2021)
BC	circMETTL3	Up	METTL3/METTL14/FTO	Writer/writer/eraser	Oncogene	circBank, m^{6}A RIP	Sponge miR-31-5p to upregulate CKD1 expression	Li et al. 2021a)
CC	circE7	Up	METTL3	Writer	Oncogene	m^{6}A RIP	Translate into E7 oncoprotein	Zhao et al. (2019)
CC	circARHGAP12	Up	IGF2BP2	Reader	Oncogene	MeRIP assay	Bind to IGF2BP2 to enhance FOXM1 mRNA stability	Ji et al. (2021)

Table 3 (continued)

Cancer	CircRNA name	Regulation	M^6A component	Fuction	Role in cancer	M^6A identification methods and databases	Main mechanisms	References
NSCLC	circNDUFB2	Down	METTL3/METTL14/IGF2BPs	Writer/writer/reader	Oncogene	MeRIP assay	Act as a scaffold by forming a TRIM25/circNDUFB2/IGF2BPs ternary complex to facilitate the degradation of IGF2BPs and trigger cellular immune responses by activating RIG-I	Li et al. (2021b)
Glioma	circDLC1	Down	METTL3	Writer	Anti-oncogene	m^6A level detection, MeRIP assay	Sponge miR-671-5p to facilitate the transcription of CTNNBIP1	Wu et al. (2022)
Sorafenib-resistant hepatocellular carcinoma	circRNA-SORE	Up	METTL3/METTL14/FTO	Writer/writer/eraser	Oncogene	SRAMP, RMBase v2.0, MeRIP assay	Sponge miR-103a-2-5p and miR-660-3p to activate Wnt/β-catenin pathway	Xu et al. (2020)
HCC	circMAP3K4	Up	IGF2BP1	Reader	Oncogene	SRAMP, MeRIP assay	Translate into circMAP3K4-455aa	Duan et al. (2022)
Radiotherapy-resistant hypopharyngeal squamous cell carcinoma	circCUX1	Up	METTL3	Writer	Oncogene	MeRIP assay	Bind to caspase 1 mRNA and inhibit its expression	Wu et al. (2021)

especially in the following aspects: (i) Detecting whether the m^6A level of m^6A-modified circRNAs in peripheral blood or other liquid biopsy samples can serve as biomarkers or not; (ii) Determining how much m^6A regulators and m^6A-modified circRNAs play essential roles in cancer therapy and offer potential therapeutic targets; and (iii) Overcoming the technical obstacles and challenges in studying m^6A-modified circRNAs. Based on previous research, we believe m^6A-modified circRNAs will advance the field of the epigenome, provide novel potential targets for cancer progression, and generate more serendipity.

Abbreviations

circRNAs: Circular RNAs; m^6A: N^6-methyladenosine; CVDs: Cardiovascular diseases; mRNAs: Messenger RNAs; ncRNAs: Non-coding RNAs; m^5C: 5-Methylcytosines; 5hmC: 5-Hydroxymethylcytosine; m^1A: N^1-methyladenosines; m^6Am: N^6, 2′-Odimethyladenosine; m^7G: 7-Methylguanine; Ψ: Pseudouridine; CDS: Coding sequence; 3′-UTRs: 3′-Untranslated regions; RBP: RNA binding protein; TSEN: TRNA splicing endonuclease; BHB: Bulge-helix-bulge motif; EcircRNAs: Exonic circRNAs; ElciRNAs: Exon–intron circRNAs; CiRNAs: Intronic circRNAs; TricRNAs: TRNA intronic circular RNAs; miRNA: MicroRNA; ceRNA: Competing endogenous RNA; IRES: Internal ribosome entry site; ITAFs: IRES-transacting factors; MTC, also named "writers": Methyltransferases complex; METTL3: Methyltransferase-like 3 protein; METTL14: Methyltransferase-like 14 protein; WTAP: Wilms Tumor 1 Associated Protein; VIRMA, also called "Virilizer" or "KIAA1429": Vir-like m^6A methyltransferase associated; RBM15/15B: RNA recognition motif 15/15B; ZC3H13: Zinc finger CCCH domain-containing protein 13; CBLL1, also known as "HAKAI": Cbl proto-oncogene-like 1; METTL16: Methyltransferase-like 16; METTL5: Methyltransferase-like 5; ZCCHC4: Zinc finger CCCH-Type containing 4; FTO, also known as "ALKBH9": Fat mass and obesity-associated protein; ALKBH5: AlkB homolog 5; αKG: α-Ketoglutaric acid; YTH: YT521-B homology; YTHDF1: YTH domain family protein 1; YTHDF2: YTH domain family protein 2; YTHDF3: YTH domain family protein 3; YTHDC1: YTH domain containing 1; YTHDC2: YTH domain containing 2; CCR4-NOT: Carbon catabolite repressor 4-negative on TATA; P-body: Processing body; eIF3: Eukaryotic initiation factor 3; eIF4E: Eukaryotic initiation factor 4E; eIF4G: Eukaryotic initiation factor 4G; PABP: Poly(A) binding protein; HNRNPC: Heterogeneous nuclear ribonucleoprotein C1/C2; HNRNPG: Heterogeneous nuclear ribonucleoprotein G; HNRNPA2B1: Heterogeneous nuclear ribonucleoprotein A2B1; HNRNP: Heterogeneous nuclear ribonucleoprotein; IGF2BP1/2/3: Insulin-like growth factor 2 mRNA-binding protein 1/2/3; FMRP: Fragile X mental retardation protein; PRRC2A: Proline-rich spiral coil 2A; NGS: Next-generation sequencing; ELISA: Enzyme-linked immunosorbent assay; RNase R: Ribonuclease R; MeRIP: Methylated RNA immunoprecipitation; RIP: RNA immunoprecipitation; qPCR: Quantitative real-time polymerase chain reaction; MeRIP-seq: Methylated RNA immunoprecipitation and sequencing; nanopore DRS: Nanopore-based direct RNA sequencing; eIF4G2: Eukaryotic translation initiation factor 4 gamma 2; eIF3A: Eukaryotic initiation factor 3A; HPV: Human papillomavirus; Hel25E: Helicase at 25E; nuclear RNA helicase URH49: ATP-dependent RNA helicase DDX39A; dead box protein UAP56: Spliceosomal RNA helicase DDX39B; YAP1: Yes-associated protein 1; HMGA2: High Mobility Group AT-Hook 2; CDR1: Cerebellar degeneration associated protein 1; Ago2: Argonaute2; RNAi: RNA interference; HRSP12: Heat-responsive protein 12; RNase P: Ribonuclease P; MRP: Mitochondrial RNA processing; CRC: Colorectal carcinoma; GEO: Gene Expression Omnibus; TCGA: The Cancer Genome Atlas; TCF7: Transcription factor 7; EMT: Epithelial-mesenchymal transition; SMAD3: Mothers against decapentaplegic homolog 3; LM: Liver metastasis; RNA-EMSA: RNA electrophoretic mobility shift assay; FISH: Fluorescence in situ hybridization; GC: Gastric cancer; DECs: Differentiated expressed circRNAs; PDGA: Poorly differentiated gastric adenocarcinoma; AKT1S1: AKT1 substrate 1; EIF4B: Eukaryotic translation initiation factor 4B; HCC: Hepatocellular carcinoma; ICGC: International Cancer Genome Consortium; HuR: Human Antigen R; MMP1: Matrix metalloproteinase 1; BC: Breast cancer; CKD1: Cyclin-dependent kinases; CC: Cervical cancer; HPV: Human papillomavirus; FOXM1: Forkhead box M1; NSCLC: Non-small cell lung cancer; TRIM25: Tripartite motif containing 25; RIG-I: Retinoic acid-inducible gene-I; CTNNBIP1: Catenin Beta Interacting Protein 1; Wnt/β-catenin: Wingless-types/beta-catenin; circMAP3K4-455aa: CircMAP3K4 translation produced a 455 amino acid protein; HPSCC: Hypopharyngeal squamous cell carcinoma; CEA: Carcinoembryonic antigen; PFS: Disease-free survival rate; OS: Overall survival rate; ICB: Immune checkpoint blockade.

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Author contributions

SQ and SJ conceived the structure of the manuscript and drafted the initial manuscript and charts. QZ collated the literature and proofread the manuscript. YX, SM, and TW provided valuable advice and participated in the final revision of the manuscript. YH checked and revised the final manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors have no conflict of interest.

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