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, N^6^-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 emerged and attracted 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. 2019; 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′-O-dimethyladenosine (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...
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 (ElciRNAs), 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, m6A-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 ElciRNAs, such as Ci-ankrd52, ElciPAIP2, and ElciEIF3J, 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.

(See figure on next page.)

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, ElciRNA, 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 (RBMB15/15B) (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) dioxygenases, 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 elf3 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<sup>6</sup>A methylation has seen a dramatic shift.

**Quantitative and semi-quantitative detection of m<sup>6</sup>A-modified circRNAs**

As a semi-quantitative method for determining the level of overall m<sup>6</sup>A-modified circRNAs, dot blot is easy to operate and time-saving, but it only can confirm the presence of m<sup>6</sup>A or compare the amount of m<sup>6</sup>A in different groups, but cannot quantify or locate m<sup>6</sup>A (Zhou et al. 2017). In addition, the m<sup>6</sup>A level detection is a colorimetric method for quantifying the overall level of m<sup>6</sup>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<sup>6</sup>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<sup>6</sup>A RIP), is a method for enriching m<sup>6</sup>A-modified circRNAs by using an anti-m<sup>6</sup>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<sup>6</sup>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<sup>6</sup>A modification sites in circRNAs**

Although most relevant methods focus on detecting m<sup>6</sup>A modifications in linear RNAs, the precise detection of m<sup>6</sup>A modification sites in circRNAs remains uncommon. Methylated RNA immunoprecipitation and sequencing (MeRIP-seq/m<sup>6</sup>A-seq), is a predominant method for detecting m<sup>6</sup>A modifications in RNAs. It mainly combines anti-m<sup>6</sup>A antibody with m<sup>6</sup>A-containing RNA fragments for NGS. The m<sup>6</sup>A-seq approach has some limitations: (i) It can only identify m<sup>6</sup>A hypermethylation enrichment regions on RNAs with a resolution of about 100nt, but cannot locate individual m<sup>6</sup>A sites; (ii) It requires a large number of total RNA samples due to its low sensitivity; (iii) Antibodies to m<sup>6</sup>A can recognize modifications similar to m<sup>6</sup>A, such as m<sup>6</sup>Am, with less specificity (Dominissini et al. 2012; Antanaviciute et al. 2017). Notably, a variety of antibody-independent methods for detecting m<sup>6</sup>A modifications have been discovered in recent years. For example, MazF PCR is a single-base m<sup>6</sup>A detection method that uses the m<sup>6</sup>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<sup>6</sup>A modification fraction at any specific RNA site. It is worth noting that both MazF PCR and ligase-dependent PCR assays for detecting m<sup>6</sup>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<sup>6</sup>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<sup>6</sup>A-related fundamental studies and clinical diagnosis (Table 1).

**Databases for predicting m<sup>6</sup>A-modified circRNAs**

The databases for predicting m<sup>6</sup>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 Deep-M6ASeq (Zhang and Hamada 2018). These databases can predict not only m<sup>6</sup>A modifications but also circRNAs with miRNA binding sites, protein-coding potential, conservations, mutations, etc. Notably, m6A2Target is a
Fig. 2 (See legend on previous page.)
A 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).

| Table 1 | Detection methods for m^6^A-modified circRNAs |
|---------|-----------------------------------------------|
| Methods                              | References                     |
| Quantitative and semi-quantitative detection | Zhou et al. (2017) |
| Dot blot                             | Ge et al. (2020)               |
| m^6^A level detection                 | Chen et al. (2021)             |
| MeRIP assay/m^6^A RIP                  | Fan et al. (2022)              |
| m^6^A-circRNA epitranscriptomic microarray | Howe et al. (2021)           |
| The detection of m^6^A modification sites | 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)             |

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 eIF4G2. 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)          |
| RMBaseV2.0 | 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)          |
M6A 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 m6A reader YTHDC1 can bind to circNSUN2 and facilitate circNSUN2 to export from the nucleus to the cytoplasm in an m6A-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 m6A 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. 2021). 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 (Hoheinz 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 epitranscriptional 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 m⁶A-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 m⁶A 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 m⁶A RNA methylation modulators (Chi et al. 2021). Besides, Liu et al. observed that KIAA1429 is negatively correlated with m⁶A-modified circDLC1 after the intersection of RNA-seq and m⁶A-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 m⁶A-regulated circDLC1 may serve as a therapeutic target for HCC (Liu et al. 2021). Additionally, MeRIP-seq, SRAMP database, and m⁶A RIP assays have confirmed that circHPS5 is highly m⁶A-modified, and METTL3 can mediate the circHPS5 formation. YTHDC1 can expedite the cytoplasmic output of m⁶A-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 m⁶A 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 m⁶A RIP assays have revealed that circMETTL3 is highly enriched in m⁶A precipitated fraction, and its expression is affected by the m⁶A 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 m⁶A-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).

m⁶A-RIP assay has confirmed that METTL3 can mediate the m⁶A 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 m⁶A motifs of circE7 can strongly inhibit E7 oncoprotein expression, implying that m⁶A-modified circE7 plays a vital role in the translation mechanism (Zhao et al. 2019). Besides, another study has found that m⁶A-modified circARHGAP12 can interact with the m⁶A 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 m⁶A-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 m⁶A 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 m⁶A-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 m⁶A level detection and MeRIP assays, Wu et al. discovered that METTL3-mediated m⁶A
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 | Function | 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 AKTI S1 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/YTHDF1   | 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) |
| Cancer | CircRNA name | Regulation | M^6A component | Function | Role in cancer | M^6A identification methods and databases | Main mechanisms | References |
|-------|--------------|------------|----------------|----------|----------------|------------------------------------------|----------------|------------|
| NSCLC | circNDUFB2   | Down       | METTL3/METTL14/IGF2BP | 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 m6A level of m6A-modified circRNAs in peripheral blood or other liquid biopsy samples can serve as biomarkers or not; (ii) Determining how much m6A regulators and m6A-modified circRNAs play essential roles in cancer therapy and offer potential therapeutic targets; and (iii) Overcoming the technical obstacles and challenges in studying m6A-modified circRNAs. Based on previous research, we believe m6A-modified circRNAs will advance the field of the epigenome, provide novel potential targets for cancer progression, and generate more serendipity.

Abbreviations
circRNAs: Circular RNAs; m6A: N6-methyladenosine; CVDs: Cardiovascular diseases; miRNAs: Messenger RNAs; ncircRNAs: Non-coding RNAs; m6C: 5-Methylcytosines; ShmC: 5-Hydroxymethylcytosine; m6A: N6-methyladenosines; m6Am: N6,N4-Dimethyladenosine; m6G: 7-Methylguanine; p: Pseudouridine; CDS: Coding sequence; 3′-UTRs: 3′-Untranslated regions; RBPs: RNA binding protein; TSCR: TRNA splicing endonuclease; BH4: Bulge-helix-bulge motif; eicircRNAs: Exonic circRNAs; EicRNAs: Exon–intron circRNAs; CRNAs: Intronic circRNAs; TricRNAs: TRNA intrinsic circRNA; mrRNA: MicroRNA; cerNA: Competing endogenous RNA, IRE: 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 m6A methyltransferase associated; RBM15/15B. RNA recognition motif protein; PRRC2A: Proline-rich spiral coil 2A; NGS: Next-generation sequencing; eIF3A: Eukaryotic initiation factor 3A; HPV: Human papillomavirus; Hel25E: RNA sequencing; eIF4G2: Eukaryotic translation initiation factor 4 gamma 2; DNase I: DNA fragmentase; qPCR: Quantitative real-time polymerase chain reaction; MeRIP-seq: Methylated RNA immunoprecipitation and sequencing; nanopore DRS: Nanopore-based direct RNA-EMSA: RNA electrophoretic mobility shift assay; FISH: Fluorescence in situ hybridization; GC: Gastric cancer; BC: Breast cancer; CK: Carcinoembryonic antigen; TNBC: Triple-negative breast cancer; bladder cancer; EMBL-EBI: European Bioinformatics Institute; ICGC: International Cancer Genome Consortium; GC: Gastric cancer; BC: Breast cancer; TCR: T-cell receptor; TRIM25: Tripartite motif containing 25; RIG-I: Retinoic acid-inducible gene-I; CTNNB1P1: Catenin Beta Interacting Protein 1; Wnt/beta-catenin: Wingless-lesions/beta-catenin; EMT: Epithelial-mesenchymal transition; SMAD3: Mothers against decapentaplegic homolog 3; TCGA: The Cancer Genome Atlas; TCF7: Transcription factor 7; IGF2BP1/2/3: Insulin-like growth factor 2 mRNA-binding protein 1/2/3; FMRP: Fragile X mental retardation 1; HNRNPA2B1: Heterogeneous nuclear ribonucleoprotein; IGF2BP1/2/3: Insulin-like growth factor 3; eIF4E: Eukaryotic initiation factor 4E; eIF4G: Eukaryotic initiation factor 4B; HCC: Hepatocellular 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. QQ collated the literature and proofread the manuscript. YY, 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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Declarations

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