Interactions of circRNAs with methylation: An important aspect of circRNA biogenesis and function (Review)

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Abstract. Circular RNA (circRNA) molecules are noncoding RNAs with unique circular covalently closed structures that contribute to gene expression regulation, protein translation and act as microRNA sponges. circRNAs also have important roles in human disease, particularly tumorigenesis and anti-tumor processes. Methylation is an epigenetic modification that regulates the expression and roles of DNA and coding RNA and their interactions, as well as of noncoding RNA molecules. Previous studies have focused on the effects of methylation modification on circRNA expression, transport, stability, translation and degradation of circRNAs, as well as how circRNA methylation occurs and the influence of circRNAs on methylation modification processes. circRNA and methylation can also regulate disease pathogenesis via these interactions. In the present study, we define the relationship between circRNAs and methylation, as well as the functions and mechanisms of their interactions during disease progression.

Contents

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1. Introduction

Gene methylation is an important epigenetic modification that involves both DNA and RNA molecules. DNA methylation mainly affects gene expression at the transcriptional level (1), whereas RNA methylation primarily regulates gene expression at the post-transcriptional level by influencing RNA stability, maturation, localization, transportation, transcription and translation (2,3). Overall, methylation modification affects numerous cellular physiological behaviors, such as neurodevelopment, immunoregulation, and cellular differentiation, and contributes to the development of various diseases (4,5).

Circular RNA (circRNA/circ) molecules are novel noncoding RNAs formed by reverse splicing of their corresponding host genes and can therefore be regulated via methylation (6). Furthermore, circRNAs can regulate gene expression and bind proteins, among other functions, and may modulate gene expression by controlling the methylation modification process (7). Therefore, fully understanding the effects of methylation modifications on the expression and function of circRNAs and an investigation into the regulation of methylation via circRNAs, are essential to determining the molecular mechanisms underlying circRNA activity and disease progression. The aim of the present review was to define the relationship between circRNAs and methylation, as well as the functions and mechanisms of their interactions during disease progression.

2. Literature search strategy

The PubMed database (pubmed.ncbi.nlm.nih.gov/) was searched using the following terms in the title or abstract: i) circRNA or circular RNA and ii) methylation or N6-methyladenosine (m6A) or m6A. The inclusion criteria

for the articles selected were that they must have been published from January 1, 2010, to September 1, 2021 and the language was English. The exclusion criteria for the articles selected were that non-original study and studies contained any duplicated data. A total of 375 papers were screened and a researcher conducted a full-text search of these publications to assess the relevance of each study. In total, 53 studies were selected for the present review.

3. DNA methylation

DNA methylation is among the earliest-discovered and most intensively studied epigenetic regulatory mechanisms. It refers to the chemical modification process, in which specific bases in DNA sequences obtain a methyl group from S-adenosyl methionine by covalent bonding via DNA methyltransferase (DNMT) activity (8,9). DNA base sites that undergo methylation include the C-5 position of cytosine, the N-6 position of adenine and the N-7 position of guanine (8,9). In mammals, the most important and only DNA methylation modification is that of the carbon atom at position C-5 of cytosine in CpG dinucleotides (8,9). DNA methylation controls gene expression by altering DNA conformation, DNA stability and DNA interactions with protein, as well as chromatin structure (8,10,11).

The methylation of DNA can be divided into *de novo* and maintenance forms, whereby the former indicates that a DNA duplex is not methylated before undergoing methylation, whereas the latter describes methylation of DNA duplexes that have been methylated previously. The methylation process is mainly mediated by DNMTs (12,13). In mammals, DNMT3a and DNMT3b perform *de novo* methylation, whereas maintenance methylation occurs via DNMT1 activity (12,13). Other proteins, such as Alpha Thalassemia/Mental Retardation Syndrome X-Linked, also contribute to methylation, assisted by accessory factors, such as the retinoblastoma protein, which function in chromosome remodeling and DNA helicase activity (14,15).

Methylated DNA can also be demethylated, which can be either a passive or an active process. In the passive process, no methylation occurs during semi-retained replication and DNA gradually becomes demethylated via 'dilution'. The active process involves demethylation via demethylases, such as DNA glycosidase and methylated CpG-binding proteins, such as methyl-CpG-binding domain protein 2 (16-18).

DNA methylation can also interact with the epigenetic regulation of histones. For example, DNA methylation leads to the removal of histone acetylation marks near the methylated site via the recruitment of histone deacetylases (19), and lysine 9 methylation on histone H3 can promote DNA methylation processes (20).

DNA methylation has important functions in mammalian development and disease processes. In particular, embryonic development involves the continuous establishment of methylation and demethylation (21,22). Methylation of CpG dinucleotide sequences in gene promoter regions can lead to silencing of gene expression (1), which can also serve an important role in disease pathogenesis. For example, via the inactivation of the tumor suppressor gene, cyclin-dependent kinase inhibitor 2A, following methylation (23-25), which is an important cause of tumorigenesis.

4. RNA methylation

RNA methylation refers to the methyltransferase catalysis chemical modification of methyl groups via selective addition of methyladenine to RNA, which primarily regulates gene expression at the post-transcriptional level (2). To date, more than 170 cellular RNA modifications have been identified in various coding and noncoding RNAs (26). m6A is the most abundant and important RNA modification in eukaryotic mRNAs, which is mainly located near stop codons and in the 3'noncoding region and is an important epigenetic modification of both mRNA and noncoding RNA (27). Methylation can affect the stability, localization, transportation, transcription, maturation and translation of RNA molecules at the post-transcriptional level, which thereby regulates gene expression (2,3).

M6A methylation of RNA occurs mainly on adenines in the DRACH sequence (m6A consensus motifs) and is regulated by methyltransferase-encoders, demethylase-decoders and m6A binding protein-readers (28). Methyltransferases, including methyltransferase-like (METTL)3, METTL14, WT1 associated protein and vir-like m6A methyltransferase-associated (KIAA1429), mainly promote methylation to regulate gene expression and function (28). Demethylases, primarily AlkB homolog 5 RNA demethylase (ALKBH5) and fat mass and obesity-associated protein (FTO), regulate gene function via demethylation (28). m6A binding proteins regulate the translation, degradation and splicing, as well as other gene functions modified by m6A, via recognizing and binding to m6A methylation sites (28).

RNA methylation has important biological functions in mammals, with roles in germ cell development, immunity and cell differentiation (29). RNA methylation is also closely related to numerous malignant diseases, including liver cancer, lung cancer, breast cancer and glioma (30).

5. Effects of methylation on circRNA biological function

Methylation is closely related to circRNAs. Ferreira *et al* (31) reported that enhancing CpG island methylation in the promoter regions of genes in human tumor cells leads to the significant downregulation of corresponding linear and circRNA transcript expression, including of tumor suppressor candidate 3, protein O-mannosyltransferase 1, attractin-like 1 and sterile α-motif domain containing 4A. These data indicated that methylation in gene promoter regions also affects circRNA expression. The most abundant epigenetic modification in eukaryotes of RNAs is m6A and numerous studies have confirmed that m6A is common in circRNAs. For example, 360 m6A-circRNAs have been detected in lung tissue in a hypoxia-induced pulmonary hypertension rat model and control tissues (32). A total of 9,382 circRNAs have been identified in lens epithelium cells from age-related cataract and control cells, 4,646 of which had m6A peaks with differing abundances (33). Furthermore, in a study systematically describing global circRNA m6A modification patterns in glioblastoma (GBM), researchers demonstrated that compared with the normal control group, there were 1,370 new m6A peaks and 1,322 missing m6A peaks in circRNAs from GBM. This study also demonstrated

that m6A levels were positively correlated with circRNA expression (34). Moreover, in an analysis of 10 upregulated and 10 significantly downregulated differentially expressed circRNAs, selected by analysis of the circRNA expression profiles from poorly-differentiated adenocarcinoma of the stomach, 6 upregulated and 8 downregulated circRNAs with high scores for prediction of m6A sites were identified, respectively (35). Analysis of m6A modification patterns in human ameloblastoma, relative to normal oral tissues, also detected 364 differentially methylated m6A sites within circRNAs, of which 22.5% were hypermethylated (36). In a study of circRNA expression profiles and m6A modification in poorly-differentiated gastric adenocarcinoma, a total of 65 differentially expressed circRNAs were detected, most of which had m6A modification, whereby the trend in m6A modification changes was generally consistent with that of circRNA expression levels (35). In another study, the number of circRNA reads recovered in m6A immunoprecipitation experiments was compared with the total input of circRNA sequence reads, leading to an estimation that ~13% of total circRNAs possess an m6A modification (37). m6A tends to occur in larger exonic regions of circRNAs and is concentrated upstream and in the middle of exonic regions, and m6A exhibits cell-specificity, for example, more than half of m6A-circRNAs detected in HeLa cells were not detected in human embryonic stem cells (38,39).

Effects of methylation on the biogenesis, transport, stability, translation and degradation of circRNA. Fig. 1 presents the use of three small interfering (si) RNAs to downregulate METTL3 expression led to the strong and specific downregulation of circ-zinc finger protein 609 (ZNF609) levels, accompanied by an increase in its unspliced precursor RNA, which indicated that decreased methylation may influence the conversion of precursor mRNA to circRNA (40). Similarly, when two siRNAs were used to downregulate YTH domain containing 1 (YTHDC1) expression levels, circ-ZNF609 levels decreased and those of its precursor transcript increased. Moreover, corresponding linear mRNA levels remained unchanged, with this phenomenon occurring regardless of cell type, which indicated that YTHDC1 influences circ-ZNF609 splicing (40). Further results from this study demonstrated that YTHDC1 expression does not affect circ-ZNF609 subcellular localization, excluding the possibility of circRNA degradation due to nuclear retention (40). In general, METTL3 and YTHDC1 influence circRNA formation by regulating m6A modification, a process that is driven by m6A modification at specific sites (39).

A study on the effects of circNOP2/Sun RNA methyltransferase 2 (NSUN2) in colorectal cancer (CRC) liver metastasis, reported that it underwent m6A modification, whereas RNA pull-down and mass spectrometry analysis indicated that there was an interaction between circNSUN2, YTHDC1, serine and arginine rich splicing factor 3 (SRSF3) and nuclear RNA export factor 1 (NXF1) (41). YTHDC1, an m6A reader, interacts with the splicing factor, SRSF3, to regulate mRNA splicing and promote binding of mRNA to SRSF3 and the canonical export receptor, NXF1, to mediate m6A-modified mRNA export and metabolism (38). This therefore indicates that m6A modification may influence circNSUN2 subcellular localization. Silencing of YTHDC1 expression significantly increases circNSUN2 levels in the nucleus, whereas overexpression of wild-type YTHDC1 can restore circNSUN2 export from the nucleus to the cytoplasm (41). RNA-fluorescence *in situ* hybridization analysis has previously demonstrated that silencing of the m6A methyltransferase, METTL3, leads to a significant increase in circNSUN2 levels in the nucleus and restores defective circNSUN2 cytoplasmic export following the overexpression of wild-type METTL3 (41). Fig. 1 presents that m6A modification of circNSUN2 regulates its cytoplasmic export.

Recognition of m6A-circRNAs via YTH domain-containing family protein (YTHDF)2 regulates the stability of the corresponding parental genes (39,42). Whether YTHDF2 can regulate circRNA stability via the recognition of m6A modified circRNAs warrants further exploration. circRNA-SORE (a circRNA upregulated in sorafenib-resistant HCC cells) activates the Wnt/β-catenin signaling pathway and induces sorafenib resistance via a microRNA (miRNA/miR) sponge mechanism, while increased circRNA-SORE levels benefit from improved stability. RNA pull-down assays demonstrated that circRNA-SORE interacts with METTL3, FTO and YTHDF1/2; an encoder, decoder and reader of m6A, respectively (43), which indicated that circRNA-SORE is associated with m6A modifications. A set of sequence-specific morpholino antisense oligonucleotides designed to target the m6A site in circRNA-SORE have been used to reduce m6A-modified circRNA-SORE in HepG2-SR cells, which led to a reduction in circRNA-SORE stability (43). Furthermore, it has been demonstrated that wild-type circRNA-SORE is more stable than m6A-mutated circRNA-SORE during actinomycin D treatment (43), indicating that circRNA-SORE undergoes m6A modification and that this improves mRNA-SORE stability, which is presented in Fig. 1. These findings led to the proposal of a novel mechanism of sorafenib resistance in patients with hepatocellular carcinoma (HCC) and suggested that both circRNA-SORE and its m6A modification may be potential drug intervention targets in patients with advanced HCC.

The m6A reader, YTHDF3, interacts with ribosomal proteins to promote mRNA translation as m6A modification enables cap-independent translation (44-46). Furthermore, a recent study has reported that circRNAs can be translated into proteins (47) and therefore m6A modification may promote circRNA translation. Jakobi *et al* (48) compared the expression profiles of circRNAs from human cardiovascular cell models and cardiac tissue with those of m6A-methylated circRNAs described in previous studies (49). This aforementioned study concluded that certain m6A-modified circRNAs function in protein translation. Furthermore, Yang *et al* (37) reported that the negative control may also initiate translation when exploring internal ribosome entry site-mediated circRNA translation. Further experiments confirmed that the short sequence containing the m6A site has an important role in this process, as well as the m6A encoders, METTL3 and METTL14, and the readers, YTHDF1, YTHDF2 and YTHDF3. Moreover, circRNA translation was demonstrated to be reduced by the m6A demethylase decoder, FTO, whereas YTHDF3 was determined to strongly interact with the translation initiation

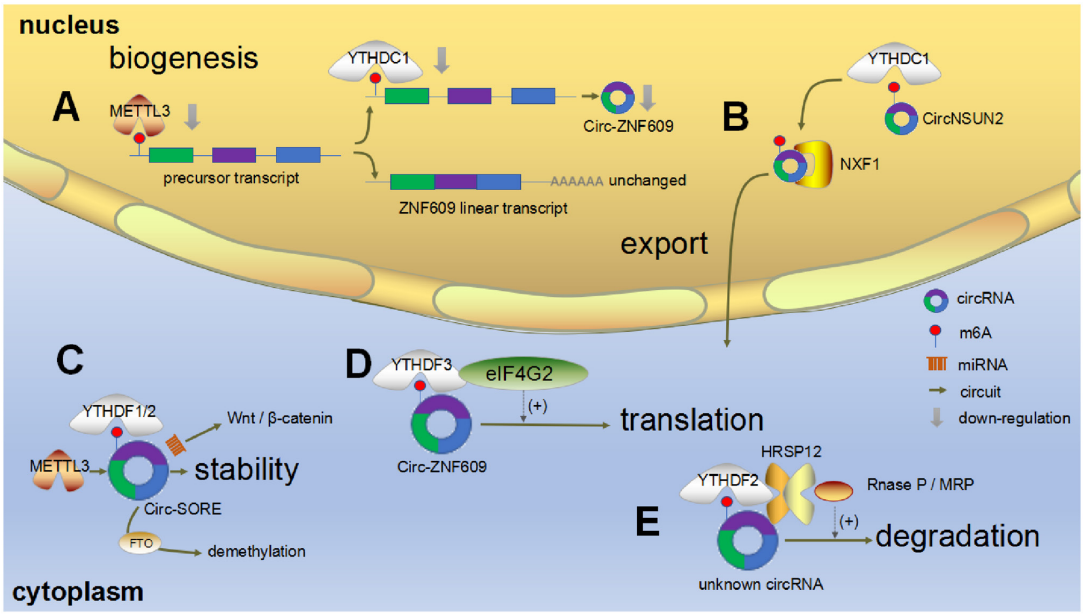

Figure 1. Effects of methylation on circRNA biogenesis, transport, stability, translation and degradation. (A) METTL3 or YTHDC1 affect circRNA generation by regulating m6A modification. (B) m6A modification regulates circNSUN2 export to the cytoplasm. (C) m6A modification improves circRNA-SORE stability. (D) YTHDF3 and eIF4G2 physically interact with endogenous circ-ZNF609 and can affect circ-ZNF609 translation levels. (E) m6A modification is involved in circRNA degradation via the YTHDF2/HRSP12/RNase P/MRP complex signaling pathway. Gray arrows represent downregulation. circRNA, circular RNA; METTL3, methyltransferase-like 3; YTHDC, YTH domain containing; m6A, N6-methyladenosine; NSUN2, NOP2/Sun RNA methyltransferase 2; YTHDF, YTH domain-containing family protein; eIF4G2, eukaryotic translation initiation factor 4-γ2; ZNF609, zinc finger protein 609; HRSP12, heat-responsive protein 12; MRP, mitochondrial RNA processing enzyme; miRNA, microRNA; FTO, fat mass and obesity-associated protein.

factor, eukaryotic translation initiation factor 4-γ2 (eIF4G2), thereby promoting translation (37). These aforementioned studies demonstrated that the transcription initiation factors, eIF4G2, YTHDF1, YTHDF2 and YTHDF3, may be involved in m6A-induced translation of circRNAs and that this process is regulated by METTL3 and METTL14. These results were confirmed by a further study on the effect of m6A modification on circ-ZNF609 translation. Fig. 1 presents although YTHDF1/2 consumption was demonstrated to have no effect on circ-ZNF609 transformation, both YTHDF3 and eIF4G2 were determined to physically interact with endogenous circ-ZNF609, with increases or decreases in these factors being reported to affect the circ-ZNF609 translation level (40).

There are two main mechanisms of mRNA degradation of m6A modification: i) The YTHDF2/C-C motif chemokine receptor 4 signaling pathway (50); and ii) the YTHDF2/heat-responsive protein 12 (HRSP12; adaptor protein)/RNase P/mitochondrial RNA processing enzyme (MRP) complex signaling pathway (51). YTHDF2 can recognize m6A and HRSP12 is an adaptor that can connect YTHDF2 and the RNase P/MRP complex. Fig. 1 presents circRNAs containing m6A are recognized by YTHDF2 in an HRSP12-dependent manner, this is followed by selective downregulation by RNase P/MRP, which indicates that m6A modification is involved in circRNA degradation via the YTHDF2/HRSP12/RNase P/MRP complex signaling pathway (52).

6. Study of methylation-modified circRNAs in disease

Studies of methylation-modified circRNA molecules in disease are summarized in Table I.

Cancer

CRC. In CRC cells, circ1662 expression increases in response to overexpression of METTL3 (53), which mediates m6A modification of the reverse complementary sequence of circ1662 to induce circ1662 expression. Furthermore, circ1662 can induce yes-associated protein 1 to translocate from the cytoplasm to the nucleus, where it activates SMAD3 to promote CRC cell invasion and migration (53).

circNSUN2 levels are elevated in tissues and sera from patients with liver metastases from CRC, indicating that it may serve a role in cancer cell metastasis. circNSUN2 levels are elevated in both the nucleus and cytoplasm of CRC cells, but particularly in the cytoplasm. Increased m6A methylation levels can promote CRC cell invasion. Moreover, a previous study demonstrated that the mutation of an m6A modification site in a plasmid for overexpression of circNUSN2, leads to the downregulation of m6A modification levels and a consequent reduction in CRC cell invasive activity. Overall, these results indicated that m6A modification of circNSUN2 is important for CRC cell invasive ability (41).

HCC. circ-deleted in liver cancer 1 (DLC1) is expressed at low levels in HCC tissues and cells and inhibits HCC proliferation and migration when it is overexpressed, which is a good prognostic indicator in patients with HCC (54). The circDLC1 molecule was identified by methylated RNA immunoprecipitation sequencing experiments, indicating that it undergoes m6A modification. Furthermore, circDLC1 is differentially expressed in response to the knockdown of the m6A methyltransferase complex, KIAA1429, which indicates that KIAA1429 regulates HCC progression via the regulation of m6A modification of circDLC1 in HCC cells (54).

Table I. M6A-modified circRNAs in disease.

First author, year	circRNA	Disease	Mechanism	Function	(Refs.)
Chen C, 2021	circ1662	Colorectal cancer	METTL3/ circ1662/YAP1/ SMAD3	Cell invasion and migration	(53)
Chen RX, 2019	circNSUN2	Colorectal cancer	Not clear	Invasion	(41)
Liu H, 2021	circDLC1	Hepatocellular carcinoma	KIAA1429/circDLC1	Cell proliferation and migration	(54)
Rao X, 2021	circ-ARL3	Hepatocellular carcinoma	HBx/METTL3/ circ-ARL3/miR-1305	Cell proliferation and invasion	(55)
Zhao J, 2019	circE7	Cervical cancer	METTL3/14/ circE7/E7 protein	Cell transformation	(56)
Chen Z, 2021	circ0000069	Cervical cancer	METTL3/ circ0000069/ miR-4426	Cell proliferation and migration	(57)
Li B, 2021	circNDUFB2	Non-small cell lung cancer	TRIM25/ circNDUFB2/IGF2BPs ternary complex	Cell growth and metastasis	(58)
Nan A, 2019	circNOL10	Lung carcinoma	circNOL10/ SCML1/HN/p53	Cell growth	(59)
Wu P, 2021	circCUX1	Hypopharyngeal squamous cell carcinoma	METTL3/circCUX1	Radiotherapy resistance	(61)
Li Z, 2021	circMETTL3	Breast cancer	METL14/ circMETTL3/ miR-31-5p-CDK1	Cell proliferation, migration and invasion	(62)
Su H, 2020	circXpo6 and circTmtc3	Pulmonary hypertension	Not clear	Not clear	(32)
Guo M, 2020 macrophage pyroptosis	hsa_circ_ 0029589	Atherosclerosis	IRF-1/METTL3/ hsa_circ_0029589		(66)
Li X, 2021	circGFRα1	Infertility	METTL14/ circGFRα1	Female germline stem cell renewal and maintenance	(67)

METTL, methyltransferase-like; YAP1, Yes-associated protein 1; SMAD3, SMAD Family Member 3; NSUN2, Sun RNA methyltransferase 2; DLC1, deleted in liver cancer 1; KIAA1429, vir-like m6A methyltransferase-associated; ARL3, ADP ribosylation factor-like GTPase 3; HBx, HBV X protein; NDUFB2, NADH:ubiquinone oxidoreductase subunit B2; TRIM, tripartite motif-containing; IGF2BP, insulin-like growth factor-2 mRNA-binding protein; NOL10, nucleolar protein 10; SCML1, Scm polycomb group protein-like 1; HN, humanin; p53, tumor protein p53; CUX1, CUT-like homeobox 1; CDK1, cyclin dependent kinase 1; Xpo6, exportin 6; Tmtc3, transmembrane O-mannosyltransferase-targeting cadherins 3; IRF-1, interferon regulatory factor 1; GFRα1, GDNF family receptor alpha 1.

circ-ADP ribosylation factor-like GTPase 3 [ARL3; also referred to as human serum albumin (hsa)_circ_0092493] is highly expressed in hepatitis B virus (HBV)-positive HCC cells and promotes their proliferation and invasion (55). The main underlying mechanism involves HBV X protein upregulation of METTL3, which increases circ-ARL3 m6A modification (55). YTHDC1A binds to m6A modified circ-ARL3 and supports its reverse splicing and biogenesis (55). Furthermore, the high expression of circ-ARL3 antagonizes the inhibitory effects of miR-1305 on oncogenes (55).

Cervical cancer (CC). Human papillomavirus type 16-derived circE7 can be translated into protein and promote the transformation of CC cells (56). The translation of this protein is independent of corresponding linear molecules and circE7 has a specific level of m6A modification (56). Silencing expression of the RNA methyltransferases, METTL3/14, can significantly inhibit circE7 expression. Mutation of a potential m6A site in the circE7 untranslated region can also significantly decrease its expression and E7 protein expression levels. However, the expression of corresponding linear molecules does not significantly change, which indicates that m6A modification promotes the expression of circE7 and its protein products, which therefore promotes CC disease progression (56).

circ0000069 promotes CC cell proliferation and migration by inhibiting miR-4426 (57). METTL3 knockdown

significantly inhibits circ0000069 m6A levels and expression and m6A modification can improve circ0000069 stability (57).

Lung cancer. In a previous study of non-small cell lung cancer, circ-NADH:ubiquinone oxidoreductase subunit B2 (circ-NDUFB2) was reported to act as a scaffold during formation of the tripartite motif-containing (TRIM) 25/circNDUFB2/insulin-like growth factor-2 mRNA-binding proteins (IGF2BPs) ternary complex, which promotes ubiquitination and degradation of IGF2BPs and inhibits non-small cell lung cancer progression (58). Levels of circNDUFB2 m6A modification affect the strength of circNDUFB2 binding to IGF2BPs, which influences the ubiquitin-ligase activity of TRIM25 for IGF2BPs (58). Furthermore, the combination of circ-nucleolar protein 10 (circNOL10) and the transcription factor Scm polycomb group protein-like 1 (SCML1) in lung cancer cells promotes SCML1 transcriptional regulation of humanin (HN), regulates the HN polypeptide family, alters various tumor signaling pathways, including p53, and inhibits lung cancer cell growth (59). Moreover, m6A methylation of pre-NOL10 in H460 and A549 cells inhibits circNOL10. Epithelial splicing regulatory protein 1 and methylated pre-NOL10 regulate circNOL10 expression together in lung cancer cells (59).

Hypopharyngeal squamous cell carcinoma (HPSCC). HPSCC is among the most common malignancies of the head and neck and is one of the malignant tumors that exhibits the worst prognosis (60). circRNAs are potential biomarkers and/or therapeutic targets in numerous types of tumor. However, the expression and function of circRNA regulation via m6A in HPSCC remains unclear. Wu *et al* (61) demonstrated that circ-CUT-like homeobox 1 (circCUX1) is upregulated in patients with HPSCC resistant to radiotherapy and that it is a predictor of poor survival. Furthermore, this study reported that METTL3 mediates circCUX1 m6A methylation and stabilizes its expression, whereas circCUX1 knockdown promotes hypopharyngeal cancer cell sensitivity to radiotherapy (61).

Breast cancer. circMETTL3 is highly expressed in breast cancer and methylated RNA immunoprecipitation (MeRIP) analysis has demonstrated that it is highly enriched in the m6A precipitation component (62). This previous study demonstrated that the downregulation of METTL14 can reduce circMETTL3 expression levels, whereas FTO reduction has the opposite effect, which indicates that circMETTL3 expression may be regulated by m6A modification levels. Furthermore, circMETTL3 was determined to promote the proliferation, migration and invasion of breast cancer cells via the circMETTL3/miR-31-5p/cyclin dependent kinase 1 (CDK1) axis (62).

Immunity and non-neoplastic disease

Immunity. Immune responses can be controlled by m6A modification (63). Chen *et al* (64) reported that m6A-modified endogenous circRNAs have important functions in dampening innate immune responses by inhibiting retinoic acid-inducible gene I (RIG-I) activation. m6A-modified exogenous circRNAs contribute to immune function by activating RIG-I-mediated innate immune responses. Furthermore, exogenous circRNAs can induce antigen-specific T and B cell activation, antibody production and antitumor immune capacity *in vivo* (64). YTHDF2 may also be essential for innate immunity suppression via recognition of m6A-modification and promotion of m6A-modified circRNA degradation (64).

Cardiovascular diseases. A previous study reported that m6A modification levels of 166 and 191 circRNAs were significantly up- and downregulated, respectively, in lung tissues in a rat model of hypoxia-induced pulmonary hypertension (HPH) compared with the controls. Moreover, m6A levels of circRNAs, which are mainly derived from coding regions, were also lower overall in HPH rats than those in the control groups (32). Gene ontology and Kyoto Encyclopedia of Genes and Genomes analyses of 76 circRNAs demonstrated their upregulation with increased m6A levels and 107 circRNAs were downregulated with reduced m6A levels. The results also demonstrated that circ-exportin 6 and circ-transmembrane O-mannosyltransferase-targeting cadherins 3 were selected as significant factors because of their enriched biding sites with target miRNAs of interest (32) and their m6A modification and downregulation were confirmed as markers of HPH in pulmonary artery smooth muscle cells and pulmonary artery endothelial cells. This aforementioned study therefore provided evidence for the potential use of m6A-circRNA as a diagnostic marker and therapeutic target in HPH (32).

Macrophage pyroptosis is among the causes of atherosclerosis and interferon regulatory factor-1 (IRF-1) can effectively promote this process (65). In one study, compared with patients with clinical presentation of chest pain and those with stable angina, hsa_circ_0029589 levels were significantly lower in patients with unstable angina and acute myocardial infarction. IRF-1 was reported to induce disease progression by downregulating hsa_circ_0029589 expression and promoting macrophage pyroptosis via METTL3 upregulation to promote hsa_circ_0029589 m6A modification (66).

Infertility. Dysregulation of m6A-modified circRNAs may affect female fertility. For example, circ-GDNF family receptor α1 (circGFRα1) is stably and abundantly expressed in female germline stem cells (FGSC) and its overexpression promotes FGSC self-renewal and maintenance (67). Furthermore, m6A modification of circGFRα1 promotes its export from the nucleus to the cytoplasm. Levels of circGFRα1 m6A modification in FGSC are also high and are regulated by METTL14 (67).

Lack of circRNAs may result in male infertility as sperm carry a wealth of evolutionarily conserved circRNAs. Considering the continuous degradation of linear RNA, the continuous enrichment of circRNAs during sperm development may provide genetic information that is translated into functional proteins (68). circRNA accumulation is associated with enhanced splicing at the m6A site and m6A modification may interfere with sperm motility by influencing circRNA expression levels (68). Lack of METTL3 is among a combination of factors in spermatogenic cells that leads to a decline in m6A levels, causing spermatogenic arrest at the meiotic phase and male infertility (68).

7. circRNAs influence disease progression via methylation regulation

The mechanisms by which circRNAs influence disease progression via methylation regulation are summarized in Table II.

Cancer

HCC. A previous study identified a total of 1,012 upregulated and 747 downregulated circRNAs in tumor samples compared with normal adjacent samples, based on genome-wide DNA methylation and RNA sequencing data from 20 patients with HCC. No significant change in the expression levels of the corresponding parental genes of 46 upregulated and 31 downregulated circRNAs in HCC tumors was detected. It was also determined that 34 (44.2%) of these 77 differentially expressed circRNAs were significantly related to changes in DNA methylation in HCC, which indicated that abnormal DNA methylation may regulate circRNA expression in HCC (69). In the same study, MeRIP-sequencing (seq) was used to identify 5-methylcytosine (m5C) sites in circRNAs in HCC and paired adjacent non-tumor tissues, and the relationship between m5C and HCC was analyzed (69). The results indicated that there may be a correlation between HCC and m5C in circRNAs. In liver cancer tissues, 51.7% of circRNAs with methylation sites had only one methylation peak, whereas the proportion was higher in adjacent tissues (69). Furthermore, hypermethylated genes were reported to exhibit lower transcriptional expression in both liver cancer and paired adjacent non-tumor tissues (69) which was confirmed in another study (70). circ-superoxide dismutase 2 is expressed at higher levels in HCC tumors than in normal liver tissues, inhibits miR-502-5p expression and rescues levels of the miR-502-5p target gene, DNMT3a, by acting as a sponge. Upregulation of DNMT3a inhibits the suppressor of cytokine signaling 3 (SOCS3) by increasing DNA methylation of the SOCS3 promoter (70), which accelerates Janus kinase 2/STAT3 signaling pathway activation, downstream of SOCS3, thereby promoting disease progression (70). Similarly, arrest of HCC disease progression caused by the overexpression of circRNA-5692 may be due to the interaction of circRNA-5692 with a methyltransferase, which reduces the methylation level of the promoter region of the tumor suppressor DAB2 interacting protein, which enhances its expression in HCC. This process is primarily mediated by circRNA-5692 acting as sponge for miR-328-5p (71).

In addition to regulating methyltransferases, circRNAs can also control gene promoter methylation by influencing the expression of other proteins associated with methylation, which can affect tumor progression. Tet methylcytosine dioxygenase 1 (TET1) mediates DNA demethylation by converting 5-methylcytosine to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine and 5-carboxycytosine (72). TET1 exerts antitumor functions in cancer cells via a variety of mechanisms, including increasing the expression of demethylated Wnt antagonists to inhibit the Wnt/β-catenin signaling pathway, or demethylating CpG sites in the promoter regions of tumor suppressor genes to promote tumor suppressor gene expression (73,74). In HCC, TET1 knockdown promotes tumor cell proliferation, migration and invasion, and circTRIM33-12 can regulate TET1 levels by acting as an miR-191 sponge. Furthermore, overexpression of circTRIM33-12 and TET1 can promote the mRNA and protein expression levels of WWC family member 3, tumor protein p53 inducible nuclear protein 1, UL16 binding protein 1 and lysine demethylase 7A via demethylation to suppress cancer. This process may also be associated with immune escape (75).

Cirrhosis is a recognized risk factor for HCC development. circ-mediator of cell motility 1 (circMEMO1) levels are significantly downregulated in HCC samples compared with cirrhotic nodules and are closely associated with overall survival and disease-free survival of patients with HCC (76). Analysis of the underlying mechanism has demonstrated that circMEMO1 can target the TET gene family by acting as a sponge of miR-106b-5p and increasing 5hmC levels, thereby regulating transcription factor 21 promoter methylation and gene expression to influence HCC progression (76). circMEMO1 can also increase HCC cell sensitivity to rafenib treatment (76).

circ-leucine rich repeats and immunoglobulin-like domains 3 (LRIG3) is significantly upregulated in liver cancer and promotes liver cell proliferation, migration and invasion and reduces apoptosis (77). circ-LRIG3 forms a ternary complex with enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) and STAT3 to promote EZH2-induced STAT3 methylation and subsequent phosphorylation, activating the STAT3 signaling pathway (77). Subsequently, activated STAT3 can directly bind to the circ-LRIG3 promoter, which increases circ-LRIG3 transcription and therefore forms a positive feedback loop (77).

Chi *et al* (78) screened for microRNAs and circRNAs differentially expressed between paired HCC tumor and normal tissues. The study using The Cancer Genome Atlas and Gene Expression Ontology databases and conducted bioinformatics analysis to construct a regulatory network of circRNA/miRNA/prognostic m6A RNA methylation modulators, which was used to screen for target circRNAs. Experimental verification demonstrated that circ-mitogen-activated protein kinase 4 promotes HCC proliferation by modulating the hsa-miR-139-5p/YTHDF1 axis (78). This aforementioned study provided a research strategy to identify circRNA targets that function in disease mechanisms via methylation regulation.

Breast cancer. Leukemia virus complex factor 1 (FLI1) promotes solid tumor progression and is highly expressed in advanced and metastatic breast cancer (79). Its role in breast cancer progression and metastasis is related to circRNA FLI1 exonic circular RNA (FECR1), which is formed from exons 2, 3 and 4 of FLI1, and can both bind to the FLI1 promoter in cis configuration and recruit TET1 via a positive feedback mechanism. It also activates FLI1 expression by binding to DNMT1 in trans configuration, thereby downregulating DNMT1 expression and inducing DNA hypomethylation to promote breast cancer metastasis (80).

Bladder cancer. IGF2BP1 can function as an m6A reader and has carcinogenic effects in cancer cells by stabilizing the methylation of oncogene mRNA molecules, including Fascin actin-bundling protein 1 (FSCN1) and MYC (81). circ-protein tyrosine phosphatase receptor type A interacts

Table II. Mechanisms of circRNA influencing disease progression via methylation regulation.

First author, year	circRNA	Disease	Methylation modification	Mechanism	Function	(Refs.)
Zhao Z, 2020	circSOD2	Hepatocellular carcinoma	DNA methylation of SOCS3	circSOD2/DNMT3a/ SOCS3/JAK2/STAT3	Cell proliferation and tumorigenesis	(70)
Liu Z, 2019	circRNA-5692	Hepatocellular carcinoma	DNA methylation of DAB2IP	circRNA-5692/miR-328/ DAB2IP	Cell proliferation, wound healing and invasion	(71)
Zhang PF, 2019	circTRIM33-12	Hepatocellular carcinoma	Demethylation of WWC3, TP53INP1,ULBP1 and JHDM1D mRNA	circTRIM33-12/miR-191/ TET1/WWC3,TP53INP1, ULBP1 and JHDM1D	Cell proliferation, migration, invasion and immune escape	(75)
Dong ZR, 2021	circMEMO1	Hepatocellular carcinoma	DNA methylation of TCF21	circMEMO1/miR-106b/ TCF21	Cell proliferation, migration and invasion and the sensitivity to sorafenib treatment	(76)
Sun S, 2020	circ-LRIG3	Hepatocellular carcinoma	Methylation of STAT3 mRNA	circ-LRIG3/EZH2/STAT3	Cell proliferation, migration, invasion and apoptosis	(77)
Chi F, 2021	circmap2k4	Hepatocellular carcinoma	Not clear	circmap2k4/miR-139-5p/ YTHDF1	Cell proliferation	(78)
Chen N, 2018	FECR1	Breast cancer	DNA methylation of FLI1	FECR1/FLI1/TET1 or FECR1/DNMT1/FLI1	Cell metastasis	(80)
Xie F, 2021	circPTPRA	Bladder cancer	m6A of FSCN1/MYC mRNA	circPTPRA/IGF2BP1/ FSCN1/MYC	Cell proliferation, migration and invasion	(81)
Huang ZM, 2021	circRNA-100284	Bladder cancer	Demethylation of HSP70	circRNA-100284/miR-217/ HSP70/Aurora-B-cell	Cell cycle transition and cell proliferation	(82)
Mo WL, 2021	hsa_circ_0072309	Non-small cell lung carcinoma	Methylation of downstream genes of FTO	hsa_circ_0072309/ miR-607/FTO	Cell tumorigenesis and invasion	(83)
Zhang Z, 2021	circRAB11FIP1	Ovarian cancer	m6A of ATG5 and ATG7 mRNA	circRAB11FIP1/FTO/ ATG5/ATG7	Autophagy flux	(84)
Wan H, 2021	circRIMS	Esophageal squamous cell carcinoma	DNA methylation of miR-613	circRIMS/miR-613	Cell proliferation	(86)
Yang P, 2021	circ-ATAD1	Endometrial cancer	DNA methylation of miR-10a	circ-ATAD1/miR-10a	Cell invasion and migration	(87)
Wu W, 2021	circFAT1	Endometrial cancer	DNA methylation of miR-21	circFAT1/miR-21	Cell stemness	(88)
Du WW, 2020	circSKA3	Glioblastoma	DNA methylation of miR-1	circSKA3/miR-1	Cell proliferation	(89)
Yang ZG, 2017	circ-Amotl1	Wound repair	DNA methylation of miR-17	circ-Amotl1/DNMT3a/ miR-17/STAT	Cell proliferation, survival, Migration and wound repair	(90)
Wang X, 2018	circIBTK	Systemic lupus erythematosus	DNA demethylation of AKT	circIBTK/miR-29b/AKT	Not clear	(93)
Zhao M, 2010	hsa_circ_0012919	Systemic lupus erythematosus	DNA methylation of CD11a and CD70	hsa_circ_0012919/ DNMT1/CD11a/CD70	Autoantibody production	(94)

Table II. Continued.

First author, year	circRNA	Disease	Methylation modification	Mechanism	Function	(Refs.)
Zhou LY, 2019	circRNA ACR	Myocardial ischemia reperfusion injury	DNA methylation of Pink1	circRNA ACR/DNMT3b/Pink	Cell autophagy	(97)
Huang R, 2020	circSTAG1	Depression	m6A of FAAH mRNA	circSTAG1/ALKBH5/FAAH	Depression-like behavior	(98)
Zhang H, 2021	circFADS2	Osteoarthritis	DNA methylation of miR-195	circFADS2/miR-195-5p	Cell apoptosis	(99)

SOD2, superoxide dismutase 2; DNMT, DNA methyltransferase; SOCS3, cytokine signaling 3; JAK2, Janus kinase 2; STAT3, signal transducer and activator of transcription 3; DAB2IP, DAB2 interacting protein; TRIM, tripartite motif-containing; TET1, Tet methylcytosine dioxygenase 1; WWC3, WWC family member 3; TP53INP1, tumor protein p53 inducible nuclear protein 1; ULBP1, UL16 binding protein 1; JHDM1D, lysine demethylase 7A; MEMO1, mediator of cell motility 1; TCF21, transcription factor 21; LRIG3, leucine rich repeats and immunoglobulin-like domains 3; EZH2, enhancer of 2 polycomb repressive complex 2 subunit; map2k4, mitogen-activated protein kinase kinase 4; YTHDF, YTH domain-containing family protein; FECR1, FLI1 exonic circular RNA; FLI1, friend leukemia virus integration 1; PTPRA, protein tyrosine phosphatase receptor type A; IGF2BP, insulin-like growth factor-2 mRNA-binding protein; FSCN1, fascin actin-bundling protein 1; MYC, MYC proto-oncogene, bHLH transcription factor; HSP70, heat shock protein 70; FTO, fat mass and obesity-associated protein; RAB11FIP1, RAB11 family interacting protein 1; ATG, autophagy related; RIMS, regulating synaptic membrane exocytosis protein; ATAD1, ATPase family AAA domain containing 1; FAT1, FAT atypical cadherin 1; SKA3, spindle and kinetochore-associated complex subunit 3; Amotl1, angiomotin-like 1; IBTK, inhibitor of Bruton tyrosine kinase; AKT, AKT serine/threonine kinase; CD, cluster of differentiation; ACR, autophagy-related circRNA; STAG1, stromal antigen 1; ALKBH5, primarily AlkB homolog 5 RNA demethylase; FAAH, fatty acid amide hydrolase; FADS2, fatty acid desaturase 2.

with the K homology domain of IGF2BP1 and prevents it from recognizing m6A-modified FSCN1 and MYC mRNAs, to inhibit bladder cancer cell proliferation, migration and invasion (81). A previous study of bladder cancer demonstrated that circ-100284 expression increases and acts as a sponge for miR-217 to induce demethylation of heat shock protein 70 (HSP70) and enhance Aurora-B activity, which accelerates cell cycle transitions and cell proliferation (82). However, the aforementioned study did not determine how miR-217 caused HSP70 demethylation (82).

Non-small cell lung carcinoma. Hsa_circ_0072309 acts as a sponge for miR-607 to upregulate FTO and promote tumorigenesis in non-small cell lung carcinoma (83). Therefore, hsa_circ_0072309 regulates downstream gene expression levels through methylation modification via the regulation of the miR-607/FTO axis to promote non-small cell lung carcinoma tumorigenesis and invasion (83).

Ovarian cancer. circ-RAB11 family interacting protein 1 induces and promotes autophagy flux in ovarian cancer cells to increase tumor cell proliferation and migration (84). This circRNA can bind to FTO mRNA and promote FTO expression, which downregulates autophagy-related gene (ATG)5 and ATG7 mRNA m6A methylation and therefore increases ATG5 and ATG7 expression levels, promoting disease progression (84).

Esophageal squamous cell carcinoma (ESCC). circ-regulating synaptic membrane exocytosis protein (circRIMS) can promote gastric cancer progression (85) and may also serve a similar role in ESCC. It has previously been reported that circRIMS was overexpressed in 60 patients with ESCC, predicting poor survival, and its levels were negatively correlated with those of miR-613, which is under-expressed in ESCC (86). In ESCC cells, overexpression of circRIMS was demonstrated to increase miR-613 methylation and reduce the inhibitory effect of miR-613 on cell proliferation (86). Furthermore, animal experiments demonstrated that circRIMS promotes tumor growth by downregulating miR-613 via methylation (86).

Endometrial cancer (EC). In an analysis comparing 60 EC and paired non-tumor tissue samples, circ-ATPase family AAA domain containing 1 (circ-ATAD1) expression was downregulated in EC tissues, whereas miR-10a was upregulated and its levels were correlated with those of circ-ATAD1 (87). Reverse transcription-quantitative PCR and methylation-specific PCR analysis, determined that circ-ATAD1 overexpression promotes miR-10a methylation and downregulates its expression, completely reversing the effect of miR-10a in enhancing EC cell invasion and migration. These results indicated that circ-ATAD1 is downregulated in EC and may function to downregulate miR-10a via methylation to inhibit EC cell invasion and migration (87). Using a similar methodology, circ-FAT atypical cadherin 1 (circFAT1) was shown to be upregulated in EC and its levels were positively correlated with those of miR-21 in EC tissues (88). Overexpression of circFAT1 increased miR-21 expression levels by reducing miR-21 gene methylation to increase cancer stem cell characteristics and promote tumor progression (88).

GBM. circ-spindle and kinetochore-associated complex subunit 3 (circSKA3) is highly expressed in breast cancer and exhibits a carcinogenic effect (89). It is also highly expressed in GBM tissues, where its levels are negatively correlated with those of miR-1 (34). Furthermore, high circSKA3 levels are significantly associated with poor survival rates of patients with GBM (34). Analysis of the underlying mechanism confirmed that overexpression of circSKA3 in GBM cells increases miR-1 methylation and decreases its expression, limiting the inhibitory effect of miR-1 on cell proliferation (34).

Wound repair, immunity and non-neoplastic disease

Wound repair. Wound healing is enhanced in mice with increased circ-angiomotin-like 1 (circ-Amotl1) expression levels. This is a result of the ectopic expression of circ-Amotl1, which leads to increased levels of the transcription factor, STAT3, and promotes its nuclear translocation, which enhances DNMT3a expression leading to miR-17 gene promoter methylation (90). Decreased miR-17-5p levels increase fibronectin, DNMT3a and STAT3 expression levels, generating a positive feedback loop (90). Together, these activities promote cell proliferation, survival and migration and enhance wound repair (90).

Immunity and immune diseases. Type three innate lymphocytes (ILC3) have key roles in innate immunity and intestinal homeostasis (91). Nuclear receptor subfamily 4 group A member 1 (Nr4a1) initiates Notch2 signal activation to help maintain ILC3 homeostasis, which depends on m6A modification of Nr4a1 mRNA by the highly expressed molecule, circ-zinc finger and BTB domain-containing protein 20 (circ-Zbtb20). Circ-Zbtb20 enhances the interaction between ALKBH5 and Nr4a1 mRNA, which indicates that circRNA and methylation may have a unique role in immune system diseases (92). For example, the expression levels of circ-inhibitor of Bruton tyrosine kinase (circ-IBTK) and miR-29b are down- and upregulated, respectively, in systemic lupus erythematosus (SLE). These expression levels correlate with SLE disease activity index score, as well as anti-double-stranded DNA and complement C3 levels in patients with SLE (93). Mechanistic studies have demonstrated that circ-IBTK may reverse miR-29b-induced DNA methylation in SLE by binding to miR-29b, which activates the AKT signaling pathway and promotes disease occurrence and development (93). Similarly, the overexpression of CD11a and CD70 in $CD4^+$ T cells contributes to the production of large amounts of autoantibodies, which induce SLE (94). Hsa_circ_0012919 upregulation is higher in $CD4^+$ T cells from patients with active SLE than in those with inactive SLE or healthy controls. Moreover, CD11a and CD70 expression and methylation levels are inversely proportional to hsa_circ_0012919 expression levels (95). Furthermore, the overexpression of DNMT1 can reverse this phenomenon, which indicates that the upregulation of hsa_circ_0012919 reduces CD11a and CD70 methylation levels by downregulating DNMT1. This induces high expression levels of these two proteins to promote SLE occurrence and progression (95).

Cardiovascular diseases. Reducing autophagy can ameliorate cardiomyocyte damage (96) and autophagy-related circRNAs (ACRs) have been identified in a myocardium ischemia-reperfusion injury model (97). PTEN-induced kinase 1 (Pink1) expression levels are significantly altered via ACR overexpression, which indicates that ACR may regulate autophagy in cardiomyocytes via Pink1. Furthermore, RNA immunoprecipitation and biotinylated probe pull-down assays demonstrated an interaction between ACR and DNMT3b, in which DNMT3b regulates Pink1 protein expression levels (97). The results of this study also demonstrated that binding of DNMT3b to the CpG region of the Pink1 promoter increases following ACR knockdown, which demonstrated that ACR can reduce the methylation level of the CpG region of the Pink1 promoter and promote Pink1 expression. This reduced the excessive activation of the autophagy signaling pathway (97).

Neurological diseases. circ-stromal antigen 1 (circ-STAG1) levels are significantly lower in hippocampus tissue from mice exposed to chronically unpredictable stress and in the peripheral blood of patients with depression. Furthermore, circ-STAG1 overexpression significantly alleviates astrocyte dysfunction and depression-like behavior caused by chronic unpredictable stress (98). circSTAG1 binds to ALKBH5 and reduces its nuclear transport, resulting in increased m6A methylation of fatty acid amide hydrolase (FAAH) mRNA and the downregulation of FAAH expression levels in astrocytes, which subsequently alleviates depression-like behavior (98).

Osteoarthritis. Lipopolysaccharide (LPS) can induce chondrocyte damage, leading to osteoarthritis. circ-fatty acid desaturase 2 (FADS2) can protect against this effect (99). circFADS2 overexpression and miR-195-5p downregulation are observed in chondrocytes following LPS treatment and the overexpression of circFADS2 increases miR-195-5p methylation, reduces miR-195-5p expression levels and downregulates apoptosis, which therefore protects chondrocytes from LPS-induced damage (99).

8. Databases related to circRNA methylation research

Databases relevant to circRNA methylation research are presented in Table III. The TRCirc (http://licpathway.net/TRCirc) database mainly focuses on the retrieval and visualization of circRNA regulatory information (100). TRCirc content includes uniform transcription factor binding site data, and H3K27ac, RNA-seq and 450k chromatin immunoprecipitation-seq array data sets. The current release of TRCirc includes data from more than 100 cell types, involving 92,375 circRNAs and 161 transcription factors, covering circRNA-related genetic and epigenetic information from 151 cell lines, including DNA methylation and super-enhancer H3K27ac signaling and expression, and supports user downloads. Currently, only human data searches are supported, providing an effective screening tool for downstream analysis of circRNAs.

The TransCirc database (http://www.biosino.org/transcirc/) (101) integrates a variety of translation-related evidence and search results intuitively present relevant evidence related to translation products. The data includes analysis of the

Table III. Databases for circRNA methylation research.

First author, year	Database	Type of data	URL	(Refs.)
Tang Z, 2019	TRCirc	DNA methylation	http://www.licpathway.net/TRCirc	(100)
Huang W, 2021	TransCirc	circRNAs with m6A modification site information	https://www.biosino.org/transcirc/	(101)
Zheng Y, 2018	m6AVar	Single nucleotide polymorphism sites that influence m6A modification	http://m6avar.renlab.org/	(102)
Luo X, 2021	RMVar	Single nucleotide polymorphism sites that influence m6A modification	http://rmvar.renlab.org/	(103)
Zhou Y, 2016	SRAMP	m6A modification site prediction on the target RNA sequence	http://www.cuilab.cn/sramp	(104)
Liu H, 2015	MeT-DB	Transcriptome methylation in mammalian cells	http://compgenomics.utsa.edu/methylation/	(105)
Liu M, 2019	Circbank	circRNA m6A modification information	http://www.circbank.cn/	(106)

translation potential of 328,080 known human circRNAs, of which there are 39,397 circRNAs with m6A modification site information.

The m6AVar (http://m6avar.renlab.org/) or RMVar (http://rmvar.renlab.org/) (102,103) databases can be used to identify single nucleotide polymorphism (SNP) sites that influence m6A modification. These databases include information on SNPs implicated in post-transcriptional regulation, miRNA binding predictions and SNPs with disease effects.

The sequence-based RNA adenosine methylation site predictor (SRAMP) database (http://www.cuilab.cn/sramp) (104) is used to predict m6A modification sites in target RNA sequences, as well as m6A modification sites in mammals. SRAMP only needs RNA sequences to run predictions, without the need to load external omics data, and is a powerful tool for m6A locus analysis where sequencing data are insufficient, such as those for long non-coding RNA and circRNA molecules.

The MethylTranscriptome (MeT-DB) database (http://compgenomics.utsa.edu/methylation/) (105) was constructed by collecting data from the Photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP)-seq and MeRIP-seq databases relating to eight m6A-related regulatory factors (FTO, KIAA1429, METTL14, METTL3, WT1-associated protein, heterogenous nuclear ribonucleoprotein C, YTHDC1 and YTHDF1) and allows visualization of specific regulatory positions using relevant sequencing data.

The Circbank database (circbank.cn/) (106) contains more than 140,000 human circRNA records, each of which has a separate detailed information page, which includes the following: i) circRNA sequence data; ii) mouse circRNAs with high homology to human circRNAs and their corresponding sequences; iii) predictive analysis of miRNA binding; iv) predictive open reading frame analysis; v) summary of mutations and polymorphism sites recorded in the Catalogue of Somatic Mutations in Cancer database (https://cancer.sanger.ac.uk/cosmic) (107); and vi) m6A modification information.

9. Conclusion

RNA methylation serves an important role in the expression, transport, stability, translation and degradation of circRNAs (108); however, to the best of our knowledge no studies have determined whether DNA methylation affects circRNA biological function. circRNAs can also regulate disease processes and may participate in immune responses by regulating proteins related to methylation modification or being modified by methylation. The interaction between circRNAs and methylation illustrates the synergistic role of epigenetics and noncoding RNA molecules in disease. Therefore, future studies focusing on the relationship between methylation and circRNAs will be important for study of mammalian development and disease. Although progress has been made in this area, further exploration is required. For example, the mechanisms underlying the effects of methylation modification on circRNA localization and expression, warrant further investigation. Moreover, to the best of our knowledge there are no relevant studies on the methylation of other basic sites in RNA, for example, the uridine modification of circRNA (another common mode of RNA methylation modification). Furthermore, the effects of circRNAs on additional cellular biological functions via methylation modification requires further elucidation, such as the metabolism, development and morphological changes of cells.

In addition to DNA methylation and RNA methylation, histone modification is also an important epigenetic process. At present, reports involving histones, methylation and circRNA are rare. For example, one study reported that circ histone-lysine N-methyltransferase eggless (Egg) inhibits histone H3 lysine 9 methylation via encoding the circEgg-P122 protein in the silkworm, *Bombyx mori* (Bm/bmo). Moreover, these results demonstrated that circEgg also promotes histone H3 lysine 9 acetylation and positively regulates gene expression of histone deacetylase Rpd, by acting as a sponge for the miRNA, bmo-miR-3391-5p (109). Furthermore,

differentially expressed circRNA may control pheochromocytoma/paragangliomas pathogenesis by regulating histone methylation, highlighting the potential role of circRNAs as biomarkers (110). However, studies concerning epigenetic modifications, such as histone methylations and circRNAs, in the context of disease, remain very limited.

The study of combination of circRNA and methylation modification faces certain future challenges. First, there is a lack of bioinformatics methods that can efficiently identify and analyze the types, sites and patterns of circRNA methylation modifications. Second, circRNAs function in brain development, due to their unique, highly stable structures (111) and methylation is also related to mammalian development (112). How to effectively study and determine the roles and underlying mechanisms of methylation and circRNA in such developmental processes will be a challenge for future research. Finally, the goal of all biomedical research is translation into clinical application. Although methylation-modified circRNAs and methylation regulated by circRNAs have been shown to influence disease processes, translation of these findings into targeted drug treatments for patients with diseases is a difficulty that needs to be overcome in the future.

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Authors' contributions

CZ, HC, WZ and DC conceived the ideas for the article and helped draft the manuscript. QY, PM and ZC performed the literature search and acquisition of data. CH and FK contributed to the analysis and interpretation of all the literature. QY, CZ and DC provided funding support. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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