# Advances in the protein-encoding functions of circular RNAs associated with cancer (Review)

WENTAO YUAN[1,2], XIAOLIN ZHANG[3] and HUI CONG[1,4]

[1]Department of Laboratory Medicine, Affiliated Hospital of Nantong University; [2]Medical School of Nantong University, Nantong, Jiangsu 226001; [3]Out-Patient Department, Dongtai Municipal People's Hospital, Yancheng, Jiangsu 224200; [4]Department of Blood Transfusion, Affiliated Hospital of Nantong University, Nantong, Jiangsu 226001, P.R. China

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**Abstract.** Circular RNAs (circRNAs) are a special class of non-coding RNAs that are widely expressed in tissues and cells. Owing to their lack of a 5'-cap and 3'-polyadenylated [poly(A)] tail, they are more structurally stable and difficult to degrade compared with linear RNA. Numerous studies published recently have suggested that circRNAs can encode peptides or proteins through cap-independent translation mechanisms and participate in the occurrence and development of cancer. In the present review, the translation mechanism underlying the encoding of proteins by circRNAs, the biological information tools that are available for predicting translation, and the identification and verification of their translational abilities are summarized and analyzed. Finally, the mechanisms associated with circRNA-encoded proteins or polypeptides in various types of cancer are summarized. In this review and its discussion on circRNAs and their coding function, we hope to provide novel perspectives and possibilities for the treatment of cancer as knowledge in this area is added to and developed in the future.

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*Correspondence to:* Mrs. Xiaolin Zhang, Out-Patient Department, Dongtai Municipal People's Hospital, 2 Kangfu West Road, Dongtai, Yancheng, Jiangsu 224200, P.R. China
E-mail: 385086556@qq.com

Dr Hui Cong, Department of Laboratory Medicine, Affiliated Hospital of Nantong University, 20 Xisi Road, Nantong, Jiangsu 226001, P.R. China
E-mail: huicjs@163.com

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## 1. Introduction

Circular RNAs (circRNAs) are a class of closed circular RNA molecules composed of upstream splicing receptors and downstream splicing donors. Owing to the lack of a 5'-cap and a 3'-polyadenylated [poly(A)] tail, circRNAs are more stable compared with linear RNA, and these molecules are not easily degraded by nucleic acid exonucleases. Originally, circRNAs were hypothesized to arise as the products of splicing errors or as intermediate products that escaped from the 'lasso' structure of introns, but given the development of high-throughput sequencing technologies and bioinformatics-based tools, circRNAs are no longer considered to be a class of RNA molecules that lack any role in humans, and their highly stable structure and remarkable tissue specificity support this notion. Conversely, an increasing number of circRNAs have been found to regulate tumor proliferation, migration, invasion, apoptosis, differentiation, metabolism, and angiogenesis (1,2) by acting as 'sponges' of microRNAs (miRNAs/miRs), interacting with RNA-binding proteins, regulating parent gene expression, and regulating alternative splicing in a number of molecular methods (Fig. 1). circRNAs regulate tumor progression through different mechanisms, and clinical trials targeting circRNA to treat cancer have shown promising therapeutic effects, showing that they play significant roles in the pathogenesis of cancer and have potential as biomarkers of cancer (3). For example, circHGS regulates the progression of bladder cancer by acting as a sponge for miR-513a-5p (4); when an intron-derived circRNA such as ci-ankrd52 was knocked down, the expression of its corresponding parent gene was also found to be significantly decreased, indicating that circRNAs may exert *cis*-effects on the expression of their parent genes (5); circPLIN2 promotes the progression of clear cell renal cell carcinoma by binding to insulin-like growth factor-2 mRNA-binding protein 2 (IGF2BP) (6); and circMbl is produced by the second exon of

the splicing factor MBL gene, and has a role in gene regulation through competing with linear splicing (7). Based on the conventional opinion, 5' and 3' untranslated regions (UTR) are essential elements for translation initiation in eukaryotic cells. Due to the absence of 5' and 3' ends, circRNAs have previously been uniformly classified as non-coding RNAs that cannot be translated (8). However, given the recent advances that have been made in this area of research, it has been shown that certain circRNAs are translated into functional proteins, which subsequently regulate the transcriptional extension of various oncogenes, and fulfill specific roles in promoting or suppressing cancer. Their protein-encoding products are considered to be potentially reliable prognostic biomarkers for certain types of tumors. For example, smo-193aa was shown to be highly expressed in glioblastoma and may be a crucial biomarker for glioblastoma development (9). In addition, breast cancer formation and development have been demonstrated to be regulated by SEMA4B-211aa and EIF6-224aa (10,11). Studies have also found that protein-encoded synthetic circRNAs can be used as therapeutics and vaccines, showing higher translational efficiency and longer stability than linear mRNA-based vaccines (12). Therefore, it has been convincingly shown that circRNA-encoded proteins exert a significant impact on cancer, and their translational function has become a research focus. The present review will subsequently focus on the progress that has been made in terms of understanding circRNA-encoded protein functions.

## 2. Translational mechanisms of circRNAs

Protein synthesis (also termed 'translation') is the name given to the process via which the genetic information stored in DNA is decoded by various catalysts that enable amino acids to be precisely located in proteins. According to the central dogma of genetics, eukaryotic mRNAs are generally translated into proteins through initiation, extension, and termination processes. The process of translation relies on the 5'-end $m^7$GpppN' ($m^7$G) cap and the 3'-end poly-A tail (13). Typically, mRNA translation relies on the 5'-cap to recognize the eukaryotic initiation factor 4F complex (eIF4F), and 43S ribosomes are recruited to direct protein synthesis. circRNA lacks a 5'-cap and a 3'-poly(A) tail, which makes it impossible to translate via this mechanism. However, mass spectrometry studies have shown that certain circRNAs can be efficiently translated into detectable peptides. Researchers have hypothesized that the presence of an internal ribosome entry site (IRES) allows it to directly recruit initiation factors and ribosomes to the circRNA independently of a cap, and subsequently, it was shown that cap-independent translation could be performed via an $m^6$A-mediated mechanism (14).

Therefore, circRNA translation requires two basic elements, namely the translation initiation element and the open reading frame (ORF). Owing to the covalent ring structure of circRNAs, special translation initiation elements are required (15). At present, the translational methods of circRNAs primarily include IRES-mediated translation and non-IRES-mediated translation. Non-IRES-mediated translation can be further divided into $N^6$-methyladenosine ($m^6$A) modification-initiated translation, rolling circle amplification (RCA) translation, and translation mediated by UTRs or other promoter elements (Fig. 2).

*IRES-mediated circRNA translation*. The IRES is a genetic element ~150-250 bp in length, and this RNA sequence folds into a structure similar to the starting tRNA, which is recognized by eIG4 γ2 (eIF4G2) and recruits ribosomes, thereby initiating protein translation. Initially, several IRES elements were identified in poliovirus and encephalomyocarditis virus, and subsequently, IRESs were also found in a variety of viruses from different viral families. IRES sequences were found to exist not only in viruses, but also in mammals, plants, and yeast, although certain differences in the functional mechanisms of different IRESs were noted (16,17). Subsequently, IRES-mediated eukaryotic translation was found to act as an emergency 'maintenance' mechanism to ensure that the body's protein requirements are met in times of stress (18). As early as 1995, it was found that an IRES sequence was also present upstream of the circRNA start codon, and in a previous study, a circRNA containing IRES element was transferred into rabbit reticulocyte lysates to obtain the predicted protein product (19). Recent studies have also demonstrated that endogenous circRNAs mediated by IRESs can be translated to produce proteins (20). For example, an IRES sequence of circ-EIF6 was identified by bioinformatics analysis, and subsequently, the putative full-length IRES sequence, as well as differentially truncated mutants, were cloned into the pGL3-Basic vector; the IRES activity was then assayed using a dual luciferase assay (11). The results showed that the putative IRES sequence had a strong ability to initiate protein translation, whereas the mutant IRES sequence could not initiate protein translation. Subsequently, red fluorescent protein (RFP) and green fluorescent protein (GFP) were cloned into the double cis-trans reporter gene construct on both sides of the putative IRES full-length sequence and the mutant sequence, respectively, to verify again whether the IRES full-length sequence could induce ribosome entry and initiate translation. The results obtained showed that both RFP and GFP were detected in cells transfected with the putative IRES plasmid under normal conditions, whereas the putative IRES plasmid only induced GFP expression when eIF4E was inhibited. Furthermore, the mutated IRES also induced significantly lower levels of GFP expression compared with the full-length IRES, suggesting that the full-length IRES sequence induced ribosome entry and initiated translation. In conclusion, IRES-mediated circRNA translation has been shown to have extended the diverse range of cellular life processes and is a novel tool of significant research value.

*$m^6$A-mediated circRNA translation*. The $m^6$A modification is the most abundant base modification in eukaryotes, with a shared sequence motif of RRACH. It is present primarily on adenine bases in the RRACH sequence, and its associated biological roles are mediated by 'writer' (methyltransferase), 'eraser' (demethylase), and 'reader' (recognition) enzymes (21). Writer enzymes are methyltransferases, including methyltransferase-like 3 (METTL3), methyltransferase-like 14 (METTL14), Wilms tumor 1 associated protein (WTAP), and Vir-like $m^6$A methyltransferase-associated (VIRMA); by contrast, AlkB homolog 5 (ALKBH5) and fat mass and obesity-associated protein (FTO) are demethylases (erasers)

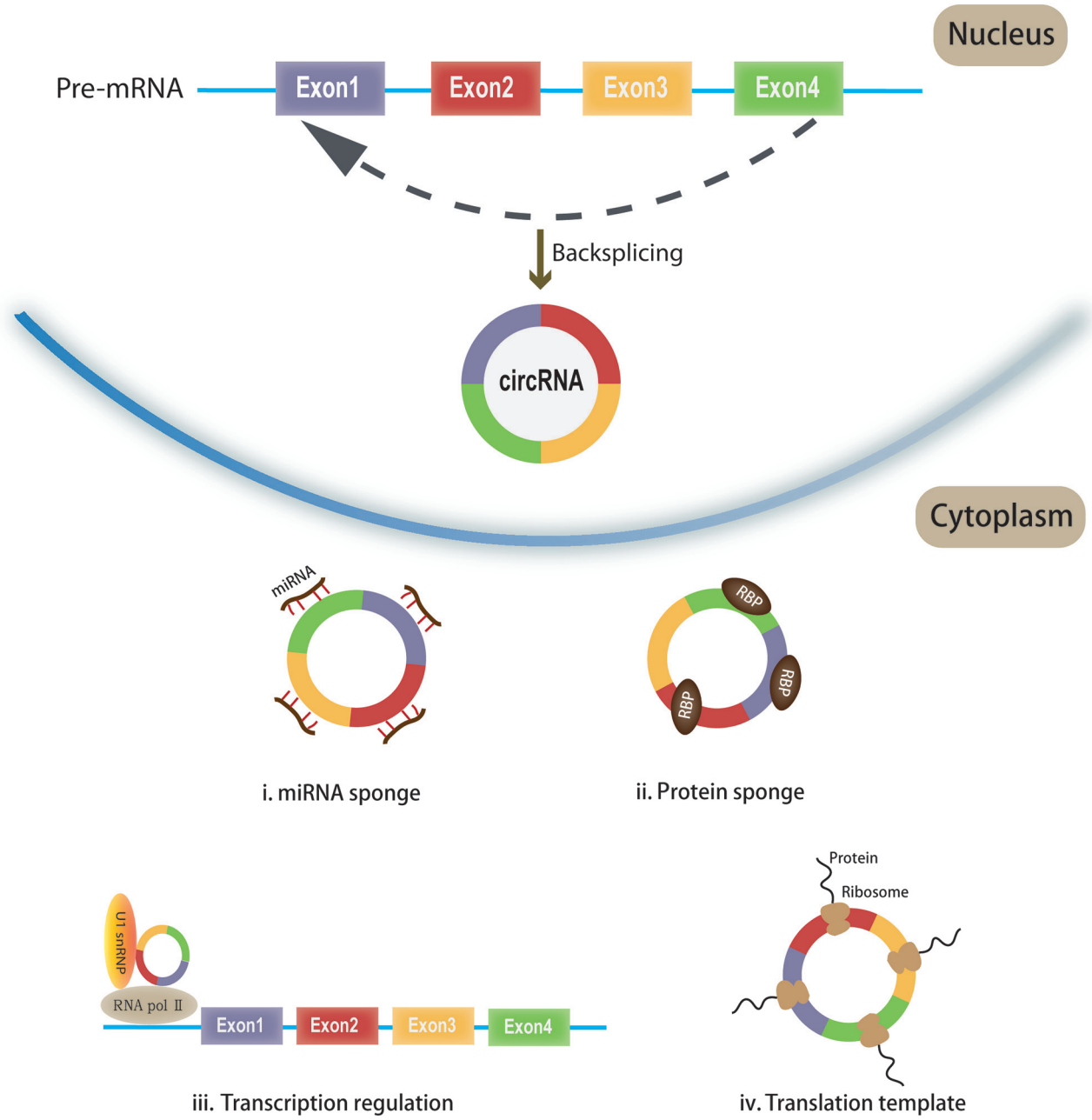


Figure 1. Biological function of circRNAs. circRNAs are formed by reverse splicing of precursor mRNA into covalently closed circular molecules. i) Sponges of miRNA molecules. circRNAs are rich in miRNA-binding sites to act as miRNA sponges. ii) Sponges of protein molecules. circRNAs bind to an mRNA-RBP. iii) Regulation of gene transcription. circRNAs interact with RNA polymerase II to regulates transcription, or EIcircRNA can interact with U1 snRNP and then bind to RNA pol II. iv) Translational function. circRNAs can be recognized by ribosomes in a cap-independent manner, which translates and encodes proteins or peptides. circRNA, circular RNA; RBP, regulated binding protein; snRNP, small nuclear ribonucleoproteins; EIcircRNA, Exon-intron circRNA; RNA pol II, RNA polymerase II; miRNA, microRNA.

that reverse methylation. m$^6$A modifications are recognized by m$^6$A-binding proteins, and it has been shown that m$^6$A-binding proteins (readers) possess YTH domain proteins, including YTH domain family proteins 1-3 (YTHDF1-3), YTH domain-containing-1 and -2 (YTHDC-1 and -2) and the heterogeneous nuclear ribonucleoprotein (HNRNP) family, including HNRNPA2B1 and HNRNPC (22). Recently, it was shown that circRNAs containing m$^6$A residues can be translated by non-cap-dependent structures. RNA immunoprecipitation (RIP) assays with m$^6$A antibodies, followed by enrichment of circRNAs and RNA sequencing (RNA-seq), has allowed the identification of all potential circRNAs that contain m$^6$A sites (23). For example, Yang *et al* (24) identified m$^6$A sites on several circRNA sequences, subsequently constructed mutants containing circRNA m$^6$A motifs, and found that circRNAs in the negative control group without an IRES could initiate translation; moreover, they identified that these circRNAs all contained an m$^6$A-modified RRACH motif near the start codon when analyzing the sequences, which also successfully confirmed that m$^6$A modification could drive the translation of circRNAs. m$^6$A-modified circRNAs are recognized by the YTHDF3 protein, which then binds to eIF4G2. eIF4G2 recruits the translation initiation factors eIF4A and eIF4B to form the translation initiation complex eIF4, which subsequently

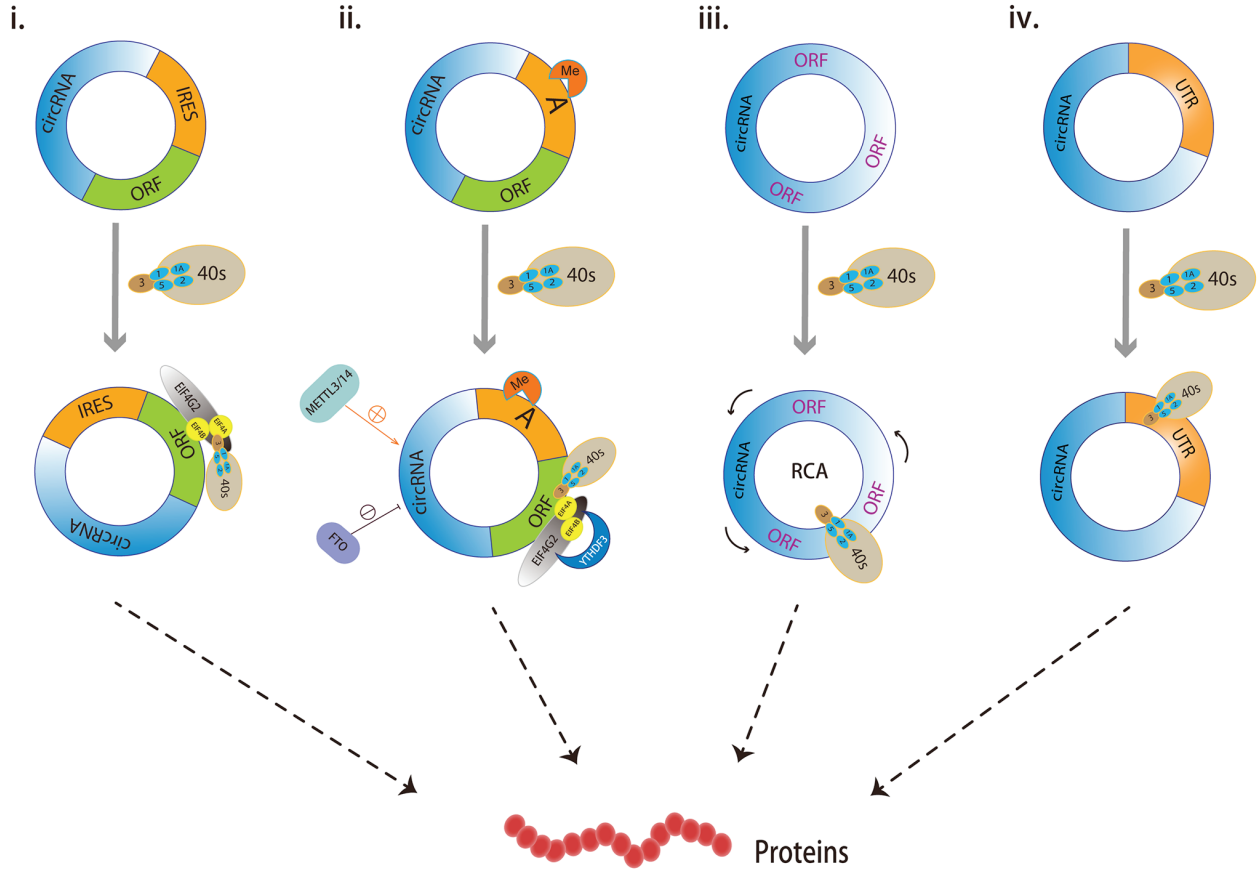


Figure 2. Common translational mechanisms of circRNAs. i) IRES-mediated circRNAs translation. The circRNAs are recognized by eIF4G2 via IRES, which recruit ribosomes for translation into proteins; ii) $m^6A$-mediated circRNA translation. circRNAs with m6A modification are recognized by the YTHDF3 protein, which then binds to eukaryotic translation initiation factor eIF4G2, which recruits translation initiation factors eIF4A and eIF4B for translation into proteins. METTL3/14 increases circRNA translation, while FTO reduces it. iii) Rolling circle amplification translation. Due to the absence of a stop codon, circRNAs have an infinite ORF, which can be translated into a circle like RCA after binding with the ribosome to produce protein expression. iv) Translation mediated by UTR elements. When certain circRNAs are reverse clipped by pre-mRNA, the clipped site is located in the UTR, resulting in the generated circRNAs having part of the same UTR sequence as the linear homologous transcripts, and the UTRs can recruit ribosomes and drive circRNA translation proteins. circRNA, circular RNA; IRES, internal ribosome entry site; RCA, rolling circle amplification; UTR, untranslated region; ORF, open reading frame.

initiates the translation of circRNA. The $m^6A$ demethylase FTO decreases the rate of circRNA translation, whereas the methyltransferase METTL3/14 increases the translation rate (8). Zhao *et al* (25) found that circE7 in carcinogenic human papillomavirus (HPV) possesses multiple potential $m^6A$ modification sites and is able to translate the E7 protein, thereby promoting the proliferation of human papilloma cells. In addition, Duan *et al* (26) showed that circMAP3K4 encodes circMAP3K4-455aa, which protected hepatocellular carcinoma (HCC) cells from cisplatin exposure driven by $m^6A$ modifications. circ-ZNF609 has been shown to be translated both in an IRES-dependent manner and through $m^6A$ modification-dependent, cap-non-dependent translation (27,28). This suggests that there are multiple mechanisms through which the translation of circRNAs may be driven. In conclusion, $m^6A$-modification-binding proteins can interact with transcription initiation factors to drive the translation of circRNAs.

*RCA translation*. In 1995, Chen and Sarnow (19) first proposed the process of RCA translation of circRNAs. RCA translation is a fast, sensitive, and constant-temperature technique of single-strand DNA amplification, which is capable of infinite single-strand amplification of circular DNA molecules. Certain circRNAs have infinite open reading frames (iORFs) due to the absence of stop codons, which allows them to be translated in a similar manner to RCAs in a rolling circle to produce high-molecular-weight proteins. For example, Das *et al* (29) found that both circASPH_219 and cicASPH_264 variants have iORFs that lack any stop codons; subsequently, they hypothesized and identified that circASPH splice variants without stop codons could be translated into macromolecular proteins with repetitive sequences through RCA translation. Gao *et al* (30) also found that circular E-cadherin (circ-E-Cad) encoded the protein C-E-Cad, producing a 254-amino-acid protein in glioblastoma. Initially, Perriman and Ares demonstrated that circRNAs could be translated *in vivo* in E. coli (31,32). Subsequently, Abe *et al* (33) constructed iORF circRNAs in E. coli and found that they were translated in a manner similar to that of the RCA reaction of polymerase, and that the circRNA molecules produced 100 times more product compared with their homologous linear mRNAs over a given period of time. In a further study by Abe *et al* (34), they sought to confirm whether circRNAs could be translated into proteins in cells. They confirmed that circRNAs synthesized *in vitro* could be translated in living human cells without internal initiation of specific elements by transferring circRNAs containing an iORF into rabbit reticulocyte lysates. Subsequently, Liu *et al* (35)

Table I. Biological information tool for circRNA encoded proteins.

| Tool | Function | Website |
|---|---|---|
| ORF Finder | All potential ORFs in circRNA sequences can be retrieved and corresponding amino acid sequences can be derived. | https://www.ncbi.nlm.nih.gov/orffinder/ |
| CircPrimer | Full-length circRNA sequences can be used to determine whether they can be encoded and to predict potential ORFs and IRES | https://www.bio-inf.cn/ |
| CPC2 | The encoding ability of circRNA can be predicted by inputting the circRNA sequence | http://cpc2.gao-lab.org/ |
| CPAT | The coding potential of circRNA was evaluated by analyzing circRNA sequence information | https://wlcb.oit.uci.edu/cpat/index.php |
| TransCirc | Intuitively present relevant evidence information of translation products and grade them | https://www.biosino.org/transcirc/ |
| RiboCIRC | A comprehensive database for predicting circRNAs translation | http://ribocirc.com/ |
| circRNADb | The first database to summarize protein-coding human circRNAs. It contains the IRES and ORFs corresponding to circRNAs and the basic characteristics of predicted peptides | http://reprod.njmu.edu.cn/cgi-bin/circrnadb/circRNADb.php |
| IRESite | All potential IRES on CircRNAs were predicted and scored and listed in the sequence | http://www.iresite.org/ |
| IRESfinder | Recognition of IRES on RNA sequences in eukaryotic cells based on k-mer feature | https://github.com/xiaofengsong/IRESfinder |
| DeepCIP | Multi-mode deep learning method is adopted to acquire circRNA IRES features more effectively | https://github.org/zjupgx/DeepCIP |
| SRAMP | Mammalian circRNA sequences were searched for the m6A motif | http://www.cuilab.cn/sramp |
| M6APred-EL | The m6A motif of circRNA in Saccharomyces cerevisiae was predicted | http://server.malab.cn/M6APred-EL/ |
| DeepM6ASeq | Based on miCLIP-Seq data, single base resolution was used to detect the m6A site | https://github.com/rreybeyb/DeepM6ASeq |
| pfam 32.0 | Potential protein domains were retrieved from ORF sequences to predict the function of protein products | https://www.ebi.ac.uk/interpro/ |

expanded further on our understanding of the translation mechanism of RCA. First, they constructed 3xFlag-tagged-circ-EGFR vectors, transfected them into 293T cells, and detected the presence of rolling translated epidermal growth factor receptor (rtEGFR) *in vivo* using either specific antibody immunoblotting or mass spectrometry methods to demonstrate the presence of 'rolling translation' *in vivo*. Next, they found that programmed-1 ribosomal frameshifting (-1PRF), mutation-induced out-of-frame stop codons could terminate infinite rolling ring translation, suggesting that the circRNAs of 'rolling translation' could also be terminated. Finally, they also identified the biological functions of certain RCA products through cell function experiments. Taken together, these studies show that the majority of the newly identified proteins translated by RCA were formerly unknown, and their underlying biological mechanisms need to be further studied.

*Translation mediated by 3'-UTRs or other primers*. Under certain conditions of stress, cap-dependent mRNA translation is usually suppressed, whereas the levels of cap-independent circRNA translation products are increased under conditions such as heat shock, hypoxia, and starvation (36). This suggests that circRNA-encoded proteins exert a role in stress responses. In addition, 3'-UTRs recruit ribosomes and drive circRNA-translated proteins. Pamudurti *et al* (37) demonstrated that circRNAs were associated with the translation of ribosomes. These researchers found that the 3'-UTR of a set of circRNAs in the head of Drosophila bound to the ribosome and detected the subsequently generated protein products. To exclude the translation interference of homologous linear mRNAs, the overexpression of eIF4E binding protein (4EBP) effectively inhibited the translation of linear mRNAs, and the protein products of their circRNAs were not affected, indicating that circRNAs can be non-dependently translated by 3'-UTR-driven caps.

## 3. Bioinformatics tools for the analysis of circRNA-encoded proteins

*Tools for predicting circRNA-encoded proteins*. CircRNAs require an ORF and translation initiation elements for their translation. The translational capacity of circRNAs has been predicted using bioinformatics tools (Table I). At present, the most commonly used prediction tools consist of a set of

methods with different objectives; the first to be discussed is ORF prediction. For this method, using circPrimer software, the user inputs the full-length sequence of the circRNA into the ORF finder to retrieve the possible ORFs and derive the corresponding amino acid sequence. Through inputting a specific circRNA, users can visualize its structure and design their own primers to verify the circRNA according to its full-length sequence, determine the presence of codable sequences according to its full-length sequence, and predict the possible ORFs and IRES. The second method that may be used (TransCirc database) is for the purposes of predicting the circRNA translational ability. Coding potential calculator 2 (CPC2) and coding-potential assessment tool (CPAT) are both able to quickly identify coding and non-coding texts from a large number of candidate texts, and to quickly and accurately assess the coding potential of RNA transcripts: The closer the assessment score is to 1, the more likely it is that the protein will be translated (38,39). The TransCirc database can integrate all types of evidence associated with translation, and the retrieval results are designed to intuitively present the information associated with the translation products, whilst also rating them (40). The RiboCIRC database is a comprehensive database for predicting cyclic RNA translation, containing predictions (and the validation) of cORF, IRES, $m^6A$, and mass spectrometry, also providing cross-species conservation evaluation of translatable circRNAs and systematic annotation of putative cyclic RNA-encoded peptides. Although this database does not include as much coding evidence as the TransCirc database, its visualization, primer design, and peptide structure analysis features are more stable and reliable than those of the TransCirc database (41). The third type of bioinformatics-based method is circRNA translation initiation mode prediction. circRNAs are generally modified by an IRES or $m^6A$ prior to the initiation of translation, and therefore it is necessary to predict whether circRNA sequences contain IRES or $m^6A$ motifs. The circRNADb database is the first database to summarize human circRNAs that encode proteins. This database contains both the IRES and the ORF information corresponding to the circRNAs, and the basic features of the predicted peptides. Alternatively, the IRESite database contains IRESs that have been experimentally validated. Through entering the sequence of a circRNA, a user can predict all the potential IRESs on the circRNAs; scores are assigned, and their corresponding positions in the sequence are listed (42,43). IRESfinder is a bioinformatics tool based on logit models, which features 19 k-mer parameters with 80% precision and 73% accuracy; this tool has a higher predictive efficiency compared with the IRESite (44). The majority of the aforementioned IRES prediction methods are based on traditional machine-learning algorithms, which are still limited as far as linear RNA IRES predictions are concerned, and at present, there are no prediction methods specifically suited for circRNA IRESs. To address this, Zhou *et al* (45) of Zhejiang University used DeepCIP, which, through a multi-modal deep learning approach, has been developed as a tool dedicated to the prediction of circRNA IRESs: This tool has enabled the study of the encoding potential of circRNAs and to more effectively capture the characteristics of circRNA IRESs. The SRAMP software is able to identify mammalian $m^6A$ sites with single-nucleotide resolution; users input mammalian circRNA sequences in order to predict the $m^6A$ motif. M6APred-EL software has been developed to predict the $m^6A$ genomic sequences of circRNAs in Saccharomyces cerevisiae and has a higher degree of accuracy compared with the other methods in terms of identification of $m^6A$ sites (46). Alternatively, DeepM6ASeq software based on miCLIP-Seq experimental data has been employed to detect $m^6A$ sites with single-base resolution and to obtain the biological characteristics that surround the $m^6A$ sites, enabling the visualization of the $m^6A$ sites. DeepM6ASeq has been shown to possess better predictive performance compared with the other machine learning methods (47). The fourth and final method to be discussed is the prediction of protein product function. The Pfam 32.0 database can be used to predict protein function: The user enters the ORF sequence of the circRNA into the Pfam 32.0 database, which subsequently can be used to search for the potential protein domain based on the ORF sequence, enabling the function of the protein product to be predicted (48).

*Identification and verification of the protein-coding abilities of circRNAs*. First, it is important to determine whether the circRNA is associated with ribosomes. To meet this aim, ribosome profiling is performed; the RNA that is not protected by a ribosome is degraded using RNase R, and the mRNA fragments that are protected by a ribosome are subsequently separated using sucrose density gradient centrifugation. Sequencing of these fragments, and screening for circRNAs bound to ribosomes, were subsequently performed (49). Second, the activity of the IRES or the $m^6A$ motif should be investigated. The luciferase activity of each plasmid compared with the luciferase activity in sea kidney is measured by constructing different luciferase reporter gene tandem plasmids. For IRES, luciferase assays revealed that the activity of the full-length IRES-induced Luc/Rluc plasmid was the highest. Regarding $m^6A$ motifs, GFP protein production, and activity could be detected by transfecting 293T cells with a short fragment containing a different copy of the $m^6A$ motif in a position prior to the circRNA reporter start codon (24). Subsequently, the ORF translational ability of circRNA should be verified. A vector containing the circRNA ORF and Flag tags was constructed, and the vector was transferred into an *in vitro* transcription-translation system. Flag antibody was subsequently used to detect the formation of the Flag fusion protein to verify whether the ORF could be encoded *in vitro*. A circRNA overexpression vector containing a Flag label was then constructed and transfected into the cells. Subsequently, a Flag label antibody was used to detect whether a protein could be generated with the same molecular weight as that determined for the protein by western blotting, to verify the translational ability of the circRNA ORF in cells. The fourth consideration is to probe with design-specific antibodies based on the ORF protein product sequence. The reliability of protein translation is detected by western blot analysis, and the product sequence is subsequently verified by immunoprecipitation and mass spectrometry to confirm the consistency of the predicted protein sequence. Fifth, it is important to verify the interaction between the circRNA and its translation initiation factors. In a previously published study, the interaction of circRNAs with eIF4G2 and IRES-transacting factors (ITAFs) associated with IRES-mediated translation (50) or YTH-domain proteins

associated with $m^6A$-mediated translation was co-verified by RIP and RNA pull-down assays (51). Finally, researchers should verify the effects of specific stimuli or proteins on circRNA translation. To meet this aim, the cells are subjected to conditions of stress such as heat shock, hypoxia, or starvation to detect whether these influence the characteristics of the circRNA-encoded proteins.

*Construction of antibodies against circRNA encoding proteins*. First, a circRNA overexpression plasmid containing a FLAG tag should be constructed by inserting a FLAG tag prior to the ORF termination codon of the circRNA, which is then transfected into 293T cells. One approach that has been used was to transfect the 293T cells, extract the total protein, and then use liquid chromatic-tandem mass spectrometry (LC-MS) to identify the specific peptide sequence of this new protein. Another method was to purify the new protein from 293T cells transfected with Co-IP and Flag antibodies, which was subsequently detected using SDS-PAGE. The new proteins were then collected for LC-MS/MS analysis to identify their specific peptide sequences. The resultant peptide sequences should match up with the predicted sequence exactly. Subsequently, by analyzing the specificity, hydrophilicity, and immunogenicity of the translation peptide, a unique fragment of the new protein is selected as the antigenic region for the preparation of the specific antibody, and the antibody is constructed to specifically target the putative circRNA translation protein (11,52).

## 4. Role of circRNA-encoded proteins in cancer

In recent years, there have been numerous reports published on newly identified circRNA-encoded proteins, and several studies have demonstrated that proteins or peptides encoded by circRNAs are able to contribute to the promotion or inhibition of cancer through a variety of different modes of action (Table II). The discovery of the proteins encoded by circRNAs has significantly expanded our understanding of the biological functions of circRNAs and provided novel perspectives for cancer therapy.

*CircRNA-encoded proteins and gastric cancer (GC)*. GC is one of the most common malignant tumors in the world and is always associated with high morbidity and mortality rates (53). Several research groups have recently discovered that circRNAs encode novel proteins in GC, and that these are crucial for the occurrence and progression of GC. According to a study by Yin *et al* (54), circAXIN1 encodes the novel protein AXIN1-295aa, which competes with the tumor suppressor adenomatous polyposis coli (APC) to stimulate the Wnt/β-catenin signaling pathway, thereby enhancing the progression of GC. The MAPK pathway is a signal transduction pathway that is well-established to be involved in cell proliferation, inflammation, and apoptosis (55,56). Jiang *et al* (57) used circRNADb to both predict and confirm that circMAPK1 encoded a novel protein, MAPK1-109aa, which exerts an inhibitory role in GC. In addition, this novel protein was able to inhibit MAPK1 phosphorylation through competitive binding with MEK1, thereby inhibiting the MAPK pathway, which demonstrated that MAPK1-109aa has a role in tumor inhibition. This suggests that circMAPK1 may serve as a therapeutic target for GC. Subsequently, Zhang *et al* (52) also employed circRNADb to predict and confirm the inhibitory effects of circDIDO1 in GC by identifying a novel protein containing 529 amino acids, DIDO1-529aa. Through mass spectrometry, immunofluorescence, immunoprecipitation, and western blotting, the encoded DIDO1-529aa protein was found to interact with polyADP-ribose polymerase 1 (PARP1) and inhibit its activity. In addition, circDIDO1 was shown to promote the ubiquitination and degradation of peroxiredoxin 2 (PRDX2), thereby inhibiting GC cell growth and invasiveness. circDIDO1 is hypothesized to be a potential prognostic biomarker and therapeutic target for GC. C-E-Cad, the protein encoded by circ-E-Cad, has been shown to exert a notable influence on the progression of glioblastoma, although its specific role in GC has yet to be fully elucidated (30). Recently, Li *et al* (58) demonstrated that circ-E-Cad-encoded C-E-Cad also exerts specific effects on GC and promotes the proliferation and migration of GC cells. In addition, Geng *et al* (59) found that circCOL6A3_030 regulates GC metastasis by encoding the protein circCOL6A3_030_198aa, which also provides a putative therapeutic direction for the diagnosis and prognosis of GC.

*circRNA-encoded proteins and colon cancer*. Colon cancer (CC) is the third most common type of cancer worldwide, and the fourth most common cause of cancer-associated death. A variety of factors, including alcohol, obesity, and genetic and epigenetic shifts, contribute to the progression of CC (60). Xiao *et al* (61) found that circPPP1R12A is upregulated in CC and carries an ORF that encodes a functional protein, namely circPPP1R12A-73aa. Relevant functional experiments confirmed that circPPP1R12A-73aa was conducive to the rapid proliferation of CC cells, and that this protein was involved in the regulation of the Hippo-Yes-associated protein (YAP) signaling pathway. Pan *et al* (62) showed that circFNDC3B may encode a novel protein containing 218 amino acids, circFNDC3b-218aa, which was shown to inhibit CC proliferation, invasion, and migration both *in vitro* and *in vivo*. Moreover, it further regulates the Snail/fructose-1,6-bisphosphatase (FBP1)/epithelial-to-mesenchymal transition (EMT) axis, thus possessing a tumor-associated role. A study by Liang *et al* (63) confirmed that circPLCE-411 encoded by circPLCE1 is involved in the regulation of NF-κB-mediated signal transduction, and that this may serve as a novel and promising therapeutic target and prognostic marker for CC. Wang *et al* (64) found that the circMAPK14-encoded protein circMAPK14-175aa was able to competitively bind with the kinase MKK14 to decrease the nuclear translocation of MAPK6, thereby promoting the ubiquitin-mediated degradation of Forkhead Box C1 (FOXC1), signifying its potential as a biomarker for the therapeutic treatment of colorectal cancer (CRC). In addition, Zhang *et al* (65) predicted that the circRNA hsa_circ_0006401 encoded a 198-amino-acid protein. An expression vector with circular transcript capability was constructed and transfected into 293T cells, whereupon it was found that the 198-aa protein was indeed encoded by hsa_circ_0006401, which has a role in CRC via promoting the proliferation and metastasis of CRC both *in vitro* and *in vivo*.

Table II. Expression and mechanism of circRNA-encoded proteins/peptides in cancer.

| First author, year | Cancer | circRNA | Encoding protein or polypeptide | Role in cancer | Change in expression | (Refs.) |
|---|---|---|---|---|---|---|
| Peng *et al*, 2021 | Gastric cancer | circAXIN1 | AXIN1-295aa | Oncogene | Upregulation | (54) |
| Jiang *et al*, 2021 | Gastric cancer | circMAPK1 | MAPK1-109aa | Tumor suppressor gene | Downregulation | (57) |
| Zhang *et al*, 2021 | Gastric cancer | circDIDO1 | DIDO1-529aa | Tumor suppressor gene | Downregulation | (52) |
| Li *et al*, 2023 | Gastric cancer | circ-E-Cad | C-E-Cad | Oncogene | Upregulation | (58) |
| Geng *et al*, 2021 | Gastric cancer | circCOL6A3_030 | COL6A3_030_198aa | Oncogene | Upregulation | (59) |
| Zheng *et al*, 2019 | Colon cancer | circPPP1R12A | PPP1R12A-73aa | Oncogene | Upregulation | (61) |
| Pan *et al*, 2020 | Colon cancer | circFNDC3B | FNDC3B-218aa | Tumor suppressor gene | Downregulation | (62) |
| Liang *et al*, 2021 | Colon cancer | circPLCE1 | circPLCE1-411 | Tumor suppressor gene | Downregulation | (63) |
| Wang *et al*, 2021 | Colon cancer | circMAPK14 | MAPK14-175aa | Tumor suppressor gene | Downregulation | (64) |
| Zhang *et al*, 2021 | Colon cancer | hsa_circ_0006401 | hsa_circ_0006401-198aa | Oncogene | Upregulation | (65) |
| Liang *et al*, 2019 | Liver cancer | circβ-catenin | β-catenin-370aa | Oncogene | Upregulation | (20) |
| Li *et al*, 2021 | Liver cancer | circARHGAP35 | ARHGAP35-1289aa | Oncogene | Upregulation | (68) |
| Li *et al*, 2022 | Liver cancer | circMRPS35 | circMRPS35-168aa | Oncogene | Upregulation | (70) |
| Duan *et al*, 2022 | Liver cancer | circMAP3K4 | circMAP3K4-455aa | Oncogene | Upregulation | (26) |
| Li *et al*, 2022 | Liver cancer | circGGNBP2 | cGGNBP2-184aa | Oncogene | Upregulation | (71) |
| Song *et al*, 2023 | Liver cancer | circZKSCAN1 | circZKSaa | Tumor suppressor gene | Downregulation | (73) |
| Wang *et al*, 2021 | Lung cancer | circASK1 | ASK1-272aa | Tumor suppressor gene | Downregulation | (75) |
| Zhao *et al*, 2022 | Lung cancer | circPPP1R12A | circPPP73R1A-73aa | Oncogene | Upregulation | (76) |
| Yang *et al*, 2018 | Glioblastoma | circFBXW7 | FBXW7-185aa | Tumor suppressor gene | Downregulation | (80) |
| Xia *et al*, 2019 | Glioblastoma | circ-AKT3 | AKT3-174aa | Tumor suppressor gene | Downregulation | (81) |
| Zhang *et al*, 2018 | Glioblastoma | circ-SHPRH | SHPRH-146aa | Tumor suppressor gene | Downregulation | (82) |
| Zhang *et al*, 2018 | Glioblastoma | circPINTexon2 | PINT87aa | Tumor suppressor gene | Downregulation | (83) |
| Wu *et al*, 2021 | Glioblastoma | circSMO | SMO-193aa | Oncogene | Upregulation | (9) |
| Gao *et al*, 2021 | Glioblastoma | circ-E-Cad | C-E-Cad | Oncogene | Upregulation | (30) |
| Liu *et al*, 2021 | Glioblastoma | circ-EGFR | rtEGFR | Oncogene | Upregulation | (35) |
| Saunders *et al*, 2023 | Glioblastoma | circ-HGF | C-HGF | Oncogene | Upregulation | (88) |
| Ye *et al*, 2019 | Breast cancer | circFBXW7 | FBXW7-185aa | Tumor suppressor gene | Downregulation | (90) |
| Li *et al*, 2020 | Breast cancer | circ-HER2 | HER2-103 | Oncogene | Upregulation | (91) |
| Li *et al*, 2022 | Breast cancer | circ-EIF6 | EIF6-224aa | Oncogene | Upregulation | (11) |
| Zhao *et al*, 2019 | Cervical cancer | circE7 | E7 | Oncogene | Upregulation | (25) |
| Gu *et al*, 2018 | Bladder cancer | circGprc5a | circGprc5a-peptide | Oncogene | Upregulation | (93) |

Table II. Continued.

| First author, year | Cancer | circRNA | Encoding protein or polypeptide | Role in cancer | Change in expression | (Refs.) |
|---|---|---|---|---|---|---|
| Tang *et al*, 2021 | Multiple myeloma | circBUB1B | circBUB1B_544aa | Oncogene | Upregulation | (95) |
| Tang *et al*, 2022 | Multiple myeloma | circHNRNPU | circHNRNPU_603aa | Oncogene | Upregulation | (96) |
| Li *et al*, 2021 | Endometrial cancer | has-circ-0000437 | CORO1C-47aa | Tumor suppressor gene | Downregulation | (97) |

circ, circular RNA; aa, amino acid; MAPK1, Mitogen-activated protein kinase 1; DIDO1, Death-inducer obliterator 1; E-Cad, E-cadherin; PPP1R12A, Protein phosphatase 1 regulatory subunit 12A; FNDC3B, Fibronectin type III domain-containing protein 3B; MAPK14, Mitogen-activated protein kinase 14; ARHGAP35, Rho GTPase-activating protein 35; MRPS35, 28S ribosomal protein S35, mitochondrial; GGNBP2, Gametogenetin-binding protein 2; MAP3K4, Mitogen-activated protein kinase kinase kinase 4; ZKSCAN1, Zinc finger protein with KRAB and SCAN domains 1; EGFR, Epidermal growth factor receptor; HGF, Hepatocyte growth factor; FBXW7, F-box/WD repeat-containing protein 7; EIF6, Eukaryotic translation initiation factor 6; HNRNPU, Heterogeneous nuclear ribonucleoprotein U.

*circRNA-encoded proteins and liver cancer.* Liver cancer is one of the most common malignant tumors in China. No obvious symptoms arise during the early stage of liver cancer, and the majority of patients are diagnosed with liver cancer at an advanced stage; as a consequence, the survival rate is low (66,67). Therefore, the search for valuable biomarkers and therapeutic targets is urgently required. Liang *et al* (20) found that circβ-catenin encoded β-catenin-370aa, which promoted the growth of hepatocellular cancer cells. β-catenin-370aa encoded by circβ-catenin may act as a decoy for glycogen synthase kinase-3β (GSK3β) to escape GSK-3β-induced β-catenin degradation; consequently, full-length β-catenin is stabilized and the Wnt pathway is activated. circRNAs can regulate the atypical functions of HCC cells through the Wnt/β-catenin pathway, which provides a novel strategy for studying the mechanism of HCC formation. Li *et al* (68) found that circARHGAP35 initiated protein translation through $m^6A$ modifications in liver cancer, and the circARHGAP35 protein interacted with the transcription factor TFI-I in the nucleus to promote the progression of cancer cells. Studies have shown that non-coding RNAs also play an important role in resistance to different cancer therapies by rewiring basic signaling pathways (69). Cisplatin chemotherapy resistance has been detected in the majority of patients with liver cancer receiving long-term chemotherapy, which is a major clinical challenge in liver cancer chemotherapy. A previous study found that circMRPS35 promoted the malignant progression of liver cancer and cisplatin resistance, and its encoded protein circMRPS35-168aa was induced by chemotherapeutic drugs to promote cisplatin resistance in liver cancer. This finding provides a novel direction for the management of drug-resistant liver cancer (70). Duan *et al* (26) found that circMAP3K4 is an upregulated circRNA with coding potential in HCC. Driven by $m^6A$ modifications, circMAP3K4 encodes the protein circMAP3K4-455aa, which was shown to promote the growth of liver cancer. The association between inflammation and tumors has also been a focus of research in recent years. A recent study identified that GGNBP2-184aa, encoded by circGNNBP2, when induced by interleukin-6, was able to activate the JAK-STAT signaling pathway, and promote the growth and proliferation of liver cancer cells (71). This study also provided a novel avenue to explore targeted therapies in the case of a liver cancer diagnosis. Sorafenib is the first-line agent in the treatment of liver cancer, as it can improve the survival rate of patients with advanced HCC (72). Song *et al* (73) showed that circZKSCAN1, and the protein it encodes, inhibit HCC progression and sensitize HCC cells to sorafenib. This finding demonstrated that the encoded protein (ZKSCAN1aa) is a potential therapeutic target and biomarker for liver cancer.

*circRNA-encoded proteins and lung cancer.* Lung cancer is the second most common malignancy in the world after breast cancer. Approximately 85% of patients with lung cancer have non-small cell lung cancer (NSCLC), of which lung adenocarcinoma (LUAD) is the most commonly observed histological subtype (74). Wang *et al* (75) showed circASK1, which encodes the recently identified protein ASK1-272aa, in LUAD was markedly downregulated, and the sensitivity of LUAD cells to gefitinib in gefitinib-resistant cells was enhanced. The protein ASK1-272aa is essential for ASK1/JNK/p38 signaling activation and mediates the chemosensitivity induction of circASK1 in LUAD. This finding highlights a novel therapeutic target for addressing gefitinib resistance in patients with LUAD. Subsequently, circPPP1R12A was shown to encode a newly identified protein, circPPP1R12A-73aa, which has been previously reported to play a catalytic role in colon cancer (61). Subsequently, Zhao *et al* (76) found that the expression of circPPP1R12A was also significantly increased in small-cell lung cancer, and its associated protein circPPP73R1A-73aa could promote the proliferation of small-cell lung cancer through the AKT pathway. Therefore, this study also provided useful insights and suggestions for the clinical treatment of NSCLC.

*circRNA-encoding proteins and glioblastoma.* Glioblastoma is the most common primary malignant tumor of the adult central nervous system, accounting for ~80% of all malignant brain tumors (77). An increasing number of studies have found

that circRNAs are differentially expressed in glioblastoma and participate in the occurrence and development of glioblastoma by acting as miRNA sponges and encoding proteins (78,79). In glioblastoma, circRNAs have been identified that may encode proteins of peptides, and these may fulfill certain roles in the occurrence and development of glioblastoma. Several studies confirmed that circRNA translation proteins regulate the occurrence and development of brain glioma (80-83). Yang *et al* (80) found that circFBXW7 encodes a novel 21 kDa protein, FBXW7-185aa, and that FBXW7-185aa inhibits the formation of malignant glioblastoma through antagonizing USP28-induced c-Myc stability by reducing the half-life of c-Myc. Furthermore, Xia *et al* (81) found that circ-AKT3 is expressed at a low level in glioblastoma, and its encoded protein, AKT3-174aa, also exerts an inhibitory role in glioblastoma. circ-SHPRH is a novel tumor-associated circRNA that has been shown to be associated with a variety of tumors, including HCC (84), GC (85), bile duct carcinoma (86), and pancreatic ductal adenocarcinoma (87), amongst others. Zhang *et al* (82) showed that circ-SHPRH, which encodes a 146-amino-acid protein (SHPRH-146aa), has an inhibitory role in tumorigenicity and that SHPRH-146aa protects full-length SHPRH from ubiquitin-proteasome degradation. Therefore, both circ-SHPRH and SHPRH-146aa may be used as potential biomarkers in glioblastoma. These researchers subsequently discovered a 53-amino-acid peptide encoded by the circRNA long intergenic non-protein-coding RNA p53-induced transcript (LINC-PINT) that was induced by long intergene non-protein-coding RNA p87, and inhibited glioblastoma cell proliferation both *in vitro* and *in vivo*. PINT87aa, which is encoded by the circular form but not linear LINC-PINT, can regulate the RNA elongation of multiple oncogenes and exert tumor inhibitory effects (83). In addition, activation of the Hedgehog signaling pathway also has a role in glioblastoma, but the sensitization mechanism has yet to be fully elucidated. Wu *et al* (9) screened circSMO with abundant Hedgehog gene expression in the database and found that it encoded a novel protein, SMO-193aa, which was later confirmed by *in vitro* experiments to be associated with Hedgehog signaling and to promote the formation of glioblastoma. Gao *et al* (30) found that the tumor suppressor gene E-Cadherin forms a circRNA (circ-E-Cad), and that the translated protein (C-E-Cad) is able to promote cancer. In addition, this research group found that circ-EGFR has an infinite cycle of ORFs, and that this particular translational product has the ability to promote the initiation of tumorigenesis in brain tumors (35). Their study also demonstrated that targeting rtEGFR leads to an improvement in the efficiency of EGFR-targeted therapy for glioblastoma. Just recently, Saunder *et al* (88) team also recently found that circHGF encoding translates the HGF protein variant, which promotes glioblastoma growth by stimulating c-MET signaling, and they boldly speculated that targeting C-HGF may have therapeutic potential for the management of GBM. Taken together, these findings on the translational function of circRNAs have provided novel ideas and insights for the treatment of glioma.

*circRNA-encoded proteins and triple-negative breast cancer (TNBC).* Breast cancer has overtaken lung cancer as the most common type of cancer worldwide, according to new figures released by the World Health Organization's International Agency for Research on Cancer (89). Approximately 15-20% of patients with breast cancer are diagnosed with TNBC, which is a subtype of breast cancer defined by the lack of expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor type 2 (Her-2). circFBXW7 has previously been reported as a tumor suppressor in glioma, which is translated into a protein product consisting of 185 amino acids (FBXW7-185aa) (80). Ye *et al* (90) also identified this circRNA in TNBC, wherein it exhibited low expression levels. Subsequently, they also explored the potential function of FBXW7-185aa in TNBC. According to the results of pre-existing studies, FBXW7-185aa has anti-cancer activity and can be used as a therapeutic target of TNBC and is a biomarker for its prognosis. Li *et al* (91) screened and verified the function of circ-HER2 in TNBC, and found that circ-HER2 encoded a protein, HER2-103, composed of 103 amino acids. Both *in vitro* and *in vivo* experiments confirmed that circ-HER and HER2-103 promote the cell proliferation, invasion and tumorigenicity of TNBC. In addition, Li *et al* (11) identified a highly expressed circ-EIF6 in TNBC through sequencing experiments and determined that circ-EIF6 had good coding ability according to polysome analysis with sucrose density gradient centrifugation. It was confirmed that circ-EIF6 encoded the protein EIF6-224aa and could promote the proliferation and invasion of TNBC cells. EIF6-224aa also promotes TNBC progression by activating the MYH9/Wnt/β-catenin pathway. In conclusion, the newly identified protein EIF6-224aa has added to our understanding of the underlying mechanisms of TNBC, and it is expected to serve as a potential prognostic and therapeutic target for patients with TNBC.

*circRNA-encoded proteins and cervical cancer.* Cervical cancer is one of the most common types of malignant tumors of the female reproductive system, and the second most common type of cancer in women worldwide. Recent studies have shown that circE7 in cervical cancer drives translation to produce E7 oncoprotein through $m^6A$ modifications. circE7 is able to inhibit cancer cell growth by reducing the level of the oncogenic E7 protein in cervical cancer cells. Since high-risk HPV detection has been established in cervical cancer screening, it remains to be determined whether circE7-translated protein can be used as a sensitive marker for high-risk HPV populations, and whether its abundance is correlated with cervical cancer prognosis (25).

*circRNA-encoded proteins and bladder cancer.* Bladder cancer is one of the most lethal cancers in the world. In recent years, numerous circRNAs have been found to be involved in a large range of biological processes in bladder cancer. These circRNAs are primarily involved in regulating the occurrence and development of bladder cancer by acting as a miRNA sponge (92). To date, several studies have discovered circRNAs with protein-coding functions in bladder cancer. Gu *et al* (93) found that circGprc5a was upregulated in bladder tumors and in bladder cancer stem cells. Subsequently, circGprc5a was found to have strong coding potential, as detected by western blotting, and the circRNA acted in a peptide-dependent manner. It was also found that a circGprc5a-peptide/Gprc5a signaling axis could be used for targeting both the bladder and bladder cancer stem cells.

This finding provides novel directions and concepts for the treatment and prognosis of bladder cancer.

*circRNA-encoded proteins and multiple myeloma*. Multiple myeloma (MM) is a malignant proliferative disease of plasma cells, often accompanied by monoclonal immunoglobulin or light chain (M protein) hyperplasia (94). Despite the clinical use of targeted drugs to improve the prognosis of patients with MM, due to drug resistance, patients are prone to relapse or refractory MM following treatment with one or more drugs, and under these circumstances, the disease becomes life-threatening and incurable. It was found that BUB1 mitotic checkpoint serine/threonine kinase B (BUB1B) was upregulated in patients with MM and it could promote the proliferation of MM cells and induce drug resistance. circBUB1B encodes a novel protein, circBUB1B_544aa, which is a noteworthy protein in that it contains a BUB1B kinase catalytic center, and it can be secreted into the bone marrow microenvironment. Both *in vitro* and *in vivo* studies have shown that circBUB1B_544aa also promotes cell proliferation and drug resistance of MM, which were attributed to the induction of activation of chromosomal instability (CIN) and centrosomal protein 170 (CEP170). These findings have demonstrated that both BUB1B and circBUB1B-544aa are promising potential therapeutic targets for MM (95). Subsequently, it was also found that circHNRNPU secreted by MM cells encodes a protein named circHNRNPU_603aa, and overexpression of circHNRNPU_603aa could promote the proliferation and cloning of MM cells both *in vitro* and *in vivo*. Moreover, circHNRNPU_603aa has the potential mechanism of regulating SKP2 exon skipping, inhibiting c-Myc ubiquitin and stabilizing c-Myc expression. The results obtained suggested that circHNRNPU_603aa may serve as a potential biomarker and therapeutic target for MM (96).

*circRNA-encoding proteins and endometrial carcinoma (EC)*. EC is the name given to a group of epithelial malignant tumors that occur in the endometrium, also known as uterine body cancer, and it is one of the three most common malignant tumors of the female genital tract. Ling *et al* (97) found through RNA sequencing that the levels of hsa-circ-0000437 were significantly reduced in EC, and that it contained a short ORF encoding the functional peptide of CORO1C-47aa. It was subsequently found that CORO1C-47aa could negatively regulate tumor development and inhibit tumor angiogenesis. The mechanism of action of CORO1C-47aa in EC cells was then explored, and it was found that CORO1C-47aa and aryl hydrocarbon receptor nuclear translocator (ARNT) competitively bound to and inhibited the co-activation of the vascular endothelial growth factor (VEGF) promoter, TACC3, thereby inhibiting VEGF expression. Taken together, the results obtained showed that the peptide CORO1C-47aa may serve as a potential target for developing effective anti-angiogenesis therapies against EC.

## 5. Concluding remarks

With the development and application of high-throughput sequencing and bioinformatics tools, an increasing number of circRNAs have been shown to have the potential to encode proteins. In the present review, we have explored the translation mechanisms associated with circRNAs, the bioinformatics tools that encode proteins, and the mechanism of action of circRNA-encoded proteins or peptides in cancer. The majority of these circRNAs have a role in inhibiting or promoting cancer through different signal transduction pathways. However, research on the function and mechanism of circRNA-encoded peptides/proteins essentially remains in its infancy, and there remain several questions to be explored and solved. For example: i) Whether there are other translational methods that are employed by circRNAs; ii) whether the bioinformatics tools still need to be further improved (many current technologies have certain limitations, and the accuracy of the prediction methods still needs to be improved); iii) whether relevant experimental techniques still need to be improved (for example, ribosome profiling and deep sequencing are not mature enough, and mass spectrometry cannot be used to detect small peptides <10 kDa in size; as a result, certain circRNA translation products cannot be detected, which presents obstacles in terms of research on the translational abilities of the circRNAs); iv) studies on these encoded peptides/proteins, up to this point, have primarily focused on cells and animals, and more human specimens, such as serum and plasma, should be considered in the future; v) the majority of the circRNAs and their translated proteins are only detected at the molecular level, and establishing their networks with other disease-causing genes in tumors needs to be further analyzed in terms of improving the strategies for clinical diagnosis and treatment; and vi) a standard nomenclature for circRNA-encoded proteins is lacking at present. The naming of the circRNAs themselves also lacks a uniform standard, since multiple circRNAs can be generated at the same gene position. Simply using the naming method of adding 'circ' as a prefix proceeded by the gene name cannot accurately define a given circRNA. Chen Lingling and his team from the Chinese Academy of Sciences, together with international experts, proposed a unified naming method for circRNAs. Chen *et al* (98) proposed to add corresponding exon serial number information to the 'circ' prefix and gene name to distinguish circRNAs with the same gene location but different exon composition. For instance, other variable splicing forms existing inside circRNA, it is recommended to add specific characters such as retention intron (RI), short (S), or long (L) to retain intron or exon information generated by internal variable splicing. For circRNAs that contain exons that are not present in existing annotations, it is recommended to use new exon (NE) as a placeholder to distinguish them. For circRNAs derived from fusion genes, experts suggest unification with the latest international fusion gene naming method, marking two fusion genes with '::', and adding the serial number information of the exon contained. The implementation of this naming convention can effectively distinguish circRNAs that originate from the same gene location but have different exon compositions. This nomenclature is based on the international standard for gene and transcript nomenclature. Of course, in addition to the need for a uniform naming convention, the provision of matching gene (and transcript) annotation information is also important to standardize circular RNA naming. In the future, it is desirable to add some additional annotation information, including the location of the circular RNA genome (and its version), the specific sequence, and a clear structural

diagram. At present, the naming method for circRNA-encoded proteins is still based on the length of the ORFs with coding potential obtained from database predictions and subsequent experimental verification. The newly identified proteins encoded by circRNAs can be efficiently identified and discriminated if the names of circRNAs are presented in a standard way. The investigation of circRNA-encoded proteins has undoubtedly begun a new era in the study of circRNAs. We hypothesize that circRNAs, and the proteins that they encode, are set to become highly intriguing clinical diagnostic targets and cancer biomarkers in the near future, as further studies on the translational function of circRNAs are published.

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**Authors' contributions**

HC and XZ provided direction and guidance throughout the preparation of this manuscript. WY collected and analyzed studies and was a major contributor in writing and editing the manuscript. HC, XZ, and WY reviewed and revised the manuscript. All authors read and approved the final manuscript. Data authentication is not applicable.

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Not applicable.

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**Competing interests**

The authors declare that they have no competing interests.

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