Mini-review

The emerging functions and roles of circular RNAs in cancer

Shibin Qu a,1, Zhengcai Liu a,1, Xisheng Yang a,1, Jingshi Zhou a, Hengchao Yu a, Rui Zhang b,*, Haimin Li a, *

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Abstract

Circular RNAs (circRNAs) are a class of single-stranded closed RNA molecules that undergo a specific backsplicing from pre-mRNA. With the application of high-throughput sequencing and bioinformatics, circRNAs are found to be widely expressed across species. Some functionally characterized circRNAs have critical roles in gene regulation through various actions, including sponging microRNAs and proteins as well as regulating transcription and splicing. Moreover, most circRNAs are aberrantly expressed in different cancer types, and some of them have been reported to play important roles in the development and progression of cancer. Given the lack of a 5′ cap structure and evidence of their ability to bind with ribosomes, circRNAs were generally considered as noncoding RNA. Notably, recent studies reported that endogenous circRNAs can be translated with a cap-independent manner, which redefines the functional roles of circRNA, further expanding the complexity of eukaryotic transcriptomes. This review aims to re-evaluate the functions and roles of circRNA from the cancer perspective. It discusses the current understanding of circRNA functions, the emerging roles of circRNA in cancer, and the challenges of future studies.

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1. Introduction

Circular RNA (circRNA) is a type of recently re-recognized RNA molecular that is renewed attention in the field of RNA. Unlike linear RNAs, circRNAs are single-stranded covalently closed circular transcripts without 5′ caps and 3′ tails [1–4]. CircRNA was first discovered in RNA viruses via electron microscopy in 1976 [5]. However, only a handful of circRNAs with little functional potential were serendipitously reported [6–10]. These circRNAs were thought to be ‘splicing noise’ or by-products of RNA processing with low abundance. CircRNAs have recently become a research hotspot, as recent studies demonstrated their roles in many biological processes [3,4,11–13]. Next-generation RNA sequencing and bioinformatic analysis have discovered that circRNAs are widely expressed across the eukaryotic tree of life [1–3,14–16]. A timeline of significant events in circRNA research is depicted in Fig. 1 [17–19].

Recently, numerous studies have shed light on the biogenesis and function of circRNAs. Circular RNA is commonly processed from precursor miRNA (pre-miRNA) backsplicing of exons [20–22]. Typically, a downstream 5′ splice site (splice donor) of an exon joins an upstream 3′ splice site (splice acceptor) to yield a circular RNA [21]. In addition, circRNA formation is often initiated with the help of reverse complementary sequences, RNA binding proteins or exon skipping [23–25]. Moreover, circRNA expression is cell type and tissue specific [26], suggesting that circRNAs might have biological functions and could be used to classify different tumor types.

Although recent studies have demonstrated that circRNAs can function as a microRNA (miRNA) or RNA binding protein sponge, and regulate splicing or transcription [27], no consensus has been achieved to date in terms of the function of circRNAs, especially the translation of circRNAs. Although some artificial circRNAs with an internal ribosome entry site (IRES) were translated [28–30], no direct biochemical evidence indicates that endogenous circRNAs are capable of protein synthesis. Hence these RNAs were typically thought to be a type of noncoding RNA [20,24,31–34]. Strikingly, in 2017, three groups found that endogenous circRNAs could produce proteins, expanding the complexity, regulation, and function of gene expression in Eukaryota [35–37]. Although many circRNAs are expressed at low levels, emerging evidence has demonstrated that...
most circRNAs are aberrantly expressed in different cancer types. Some circRNAs play important roles in multiple aspects of biology and disease, particularly in cancer [23,24].

In this review, we outline the current knowledge on the functions of circRNA, and describe the emerging roles of circRNAs in cancer. We also highlight the potential challenges for future study.

2. Emerging functions of circular RNAs

Considering the low expression of circRNA, these RNAs were overlooked and thought to lack functional significance. However, recently, circRNAs have attracted widespread attention from biological researchers, given that researchers have revealed important functional roles of various circRNAs at multiple levels (Fig. 2).

2.1. CircRNAs serve as miRNA sponges or competing endogenous RNA

The competing endogenous RNA (ceRNA) hypothesis posits that transcripts containing shared miRNA binding sites can affect miRNA activity through sequestration, thereby upregulating miRNA target expression [38]. The ceRNA hypothesis mainly involved three types of RNAs crossing talk to each other, including mRNA, transcribed pseudogenes and long noncoding RNA (lncRNA) [39]. These RNAs communicate through a new “language” mediated by miRNA response elements (MREs) to maintain dynamic balance to regulate cellular homeostasis. Now, the members of ceRNA are further expanded to include circRNA [34].

Is miRNA sponge achieved by circRNA a general feature? Using a bioinformatic approach, some studies found that no other circRNAs that characterize ciRS-7 were identified as a candidate to act as a strong sponge, and few circRNAs exhibit the properties of miRNA sponges [33,40,41]. However, with the advance of circRNA research, increasing evidence has demonstrated that ciRS-7 and circ-Sry are not an isolated example of circRNAs with ceRNA effects. For example, in a mouse model, circRNA HRCR, a heart-related circRNA named mm9-circ-012559, functions as a miR-223 sponge to regulate cardiac hypertrophy and heart failure induced by isoproterenol and transverse aortic constriction [42]. CircMFACR, another circRNA isolated from heart, regulates the apoptotic pathway in cardiomyocytes by directly sequestering miR-652-3p [43]. In MDA-MB-231 human breast cancer cells, circ-Foxo3, contains 1435 nucleotides (hsa_circRNA_104170) and can bind to eight miRNAs to inhibit the tumor growth and angiogenesis [44]. Several groups reported that circHIPK3 is abundant and derived from exon2 of the HIPK3 gene. Moreover, circHIPK3 acts as a sponge to multiple miRNAs, including miR-124, miR-558 and miR-30 family [45-47]. Other studies have found that circCCDC66 acts as a miRNA sponge to regulate myc miRNA through sponging miRNA-33b and miR-93 [48], and that circRNA_100290 may work as a ceRNA to protect CDK6 expression from attack by members of miR-29b family [49]. Another circRNA, circPVT1, binds to and blocks let-7 activity to prevent senescence [50]. CircMT01 can serve as a sponge of miR-9 [51]. Finally, loss of a mammalian CDR1as in vivo causes miR-7 and miR-671 deregulation and affects brain function [52]. Interestingly, one study found that circRNAs have a decreased single nucleotide polymorphisms (SNP) density at predicted miRNA target sites, indicating that many of these functional sites are under similar selective pressure as miRNA binding sites within 3’ UTRs [53]. Taken together, these findings support the idea that the function of circRNAs as miRNA sponges may be a general phenomenon. These data also provide a pathway to predict the noncoding function of enigmatic circRNA by predicting putative miRNA binding sites.
2.2. CircRNAs bind and sequester proteins

CircMbl is derived from the backsplicing of the second exon of the splicing factor muscleblind (MBL) [32]. It contains the start codon of the main coding sequence, suggesting the potential for translation. However, considering that researchers might pay more attention to circRNA biogenesis, they identified numerous MBL binding sites both in the sequence of circMbl and in the flanking intron of the second exon of mbl. CircMbl can sequester excess MBL to regulate the production balance of the mbl and circMbl [32]. In addition, exon-intron circular RNAs (ElciRNAs), such as circEIF3J and circPAIP2, can interact with RNA polymerase II (Pol II) to promote the expression of the parental gene [54].

Certain circRNAs also likely sequester proteins completely to block the protein effects by working as competing elements. An interesting example is that circ-Foxo3 was mainly detected in cytoplasm, where it can interact with senescence-related proteins ID1 and E2F1 and stress-related proteins HIF1a and FAK [55]. Thus, these interactions arrests these transcription factors in the cytoplasm, and consequently inhibits their biological activities. Moreover, circ-Foxo3 inhibits cell cycle progression via the formation of the circ-Foxo3-p21-CDK2 ternary complex [56]. It remains unclear whether circRNAs serve as a molecular scaffold to mediate the interaction with different proteins. Schneider and others identified a set of IMP3-associated circRNAs via IMP3-co-immunoprecipitated RNA and RNA-seq [57]. It would be interesting to investigate whether these circRNAs can bind and modulate the activity of IMP3. Another circRNA, circPABPN1, competitively binds to HuR to prevent HuR binding to PABPN1 mRNA, subsequently suppressing PABPN1 translation [58]. One interesting possibility is that mRNAs, lncRNAs, circRNAs and other molecules may also talk to each other using RNA binding protein shared response elements in the cytoplasm, perhaps analogous to ceRNA.
2.3. CircRNAs regulate transcription or splicing

Given that numerous linear transcripts that contain retained introns are located in the nucleus through nuclear restriction and nonsense-mediated mRNA decay in the cytoplasm [59], circRNAs circularized with retained introns are largely restricted to the nucleus, including circular intronic RNAs (ciRNA) [60] and ElciRNAs [54]. However, it is unclear how these circRNAs are exported or restricted to the nucleus, and the process may be similar to that for linear transcripts that contain retained introns.

CiRNAs are derived from introns, escape debranching, and depend on a consensus sequence containing a 7-nt GU-rich element close to the 5′ splice site and an 11-nt C-rich element near the branchpoint site for their formation [60]. However, the mechanism of how the consensus motif is resistant to debranching remains unclear. In addition, ciRNAs are abundant in the nucleus and regulate the expression of their parent gene. Particularly, ci-ankr52 accumulates at its sites of transcription and interacts with RNA Pol II to modulate its transcriptional activity [60].

It would be interesting to investigate whether ciRNA could bind to U1 snRNPs to promote RNA Pol II elongation in studies analogous to the previous studies [59]. Another study also discovered that a special subclass of circRNAs associate with Pol II termed ElciRNAs [54]. Knockdown of circEIF3J and circPAIP2 reduced EIF3J and PAIP2 transcription levels, respectively. Specifically, ElciRNAs binds U1 snRNA at the 5′ splice site of their retained introns and then interact with the Pol II to promote transcription of their parental genes in cis [54]. Intriguingly, both ElciRNAs and ciRNAs also localize to other sites in the nucleus, indicating that they may have other potential regulatory roles, such as roles in trans. Together, these findings indicate that nuclear circRNAs are involved in transcriptional regulation. Other partners involved in the formation of ciRNA and ElciRNA and how ciRNA and ElciRNA play roles in the biological process remain uncharacterized.

CircRNAs are generally generated from exons of pre-mRNA via backsplicing. Thus, the formation of circRNAs seems to affect the alternative splicing of such pre-mRNA, potentially producing circRNA and a corresponding linear transcript. If the circRNA contains the translation start site of host gene, it might leave a non-coding linear RNA without a start codon [20]. Alternatively, the processing of circRNAs may disrupt the integrity of original mature linear RNA, leaving a fragmentary RNA that is unable to be translated [61] or that produces an inactive protein. This processing was proposed to act as mRNA traps [62] and may result in alteration in gene expression. In addition, circularization and splicing compete against each other. As previously described, when the MBL protein is in excess, it promotes circMBl generation via binding its flanking introns, thus decreasing the production of mbl [32]. Then, this circMBl binds to the excess MBL protein to maintain the balance between circRNA biogenesis and canonical splicing. However, given that circRNA biogenesis is typically less efficient than linear RNA, it seems that most circRNAs have a limited impact on linear RNA expression through competitive splicing.

2.4. CircRNAs can be translated

Some early studies reported that most of circRNAs are not associated with polysomes via ribosome foot printing and polysome gradient analysis [1,40,41,57]. Given that circRNAs lack both a 5′ cap structure and a poly(A) tail, they were considered to fail to undergo translation via cap-dependent mechanisms. Moreover, there was no direct evidence for translation. Thus, circRNAs were generally regarded as noncoding RNA [27–30,47]. However, considering that most circRNAs are generated from coding genes and contain complete exons, it would be a natural possibility that circRNAs could be translated. Indeed, engineered circRNAs with an IRES generate proteins in vitro [28] or in vivo [30]. Some circRNAs with an IRES or open reading frame (ORF) have potential to allow for translation via bioinformatic analysis [63,64]. However, recently, everything old is new again. The world of circRNA has undergone tremendous changes. Three groups have now provided strong evidence to suggest that specific endogenous circRNAs may be used as templates for protein synthesis [35–37].

Legnini et al. used RNAi-based high-content functional genomic screening to identify one circRNA that is generated from circuarlization of the second exon of ZNF609 gene and is associated with myoblast proliferation [35]. Circ-ZNF609 contains a 753-nt ORF that bypasses the backsplicing junction. Circ-ZNF609 is associated with polysomes and encodes a protein in a splicing-dependent and cap-independent manner [35]. Considering that the circular template exhibited considerably low translational efficiency [35], which is consistent with cap-independent translation, the regulation of cap-independent translation is often used to cope with multiple cellular stresses with fast responses at the protein levels [65]. Interestingly, circ-ZNF609 translation can be activated by heat shock [35]. As previously reported, cap-acting elements, such as N6-methyladenosine (m6A) modifications, are involved in the response of cellular stresses to promote translation initiation [66]. Notably, m6A exhibits multifaceted effects on translation. A m6A modification site in the 5′ UTR was previously found to promote cap-independent translation initiation through a YTHDF2-protection mechanism in the heat shock response [67,68], whereas a m6A modification site in 3′ UTR was reported to increase translation efficiency via mediation by YTHDF1 [69]. Along this line, they also found that circ-ZNF609 is highly methylated [35], suggesting m6A modification may have an impact on the circRNA translational activity. However, the specific regulatory mechanism has not been explored to date.

To verify the potential ability of translation of circRNAs, Pamudurti and his co-workers recycled the ribosome foot printing datasets to identify ribosome-protected fragments encompassing the backsplicing junction. They found that some circRNAs, named ribo-circRNAs, are associated with translating ribosomes and are evolutionarily conserved [36]. Furthermore, 40% of ribo-circRNAs were predicted to share the start codon with the host gene. One circRNA produced from the mbl locus, termed circMbl, encodes a protein detected by mass spectrometry [36]. These findings provide strong evidence that some endogenous circRNAs can generate protein. Furthermore, Legnini and Pamudurti also found that a specific sequence in the circRNA UTR (the region located between upstream of the circRNA start codons and downstream of the circRNA stop codons) worked as IRES-like activity to drive circRNA translation [35,36]. However, further studies are needed to reveal the specific features of the functional element.

As Panda and Legnini previously mentioned [27,35], it would be of interest to test whether m6A modification is involved in cap-independent circRNA translation. Indeed, Yang et al. discovered that circRNAs contain extensive m6A modifications, and that a single m6A motif is sufficient to drive translation initiation [37]. Notably, this translation from circRNA requires eukaryotic translation initiation factor elf4G2 and m6A reader YTHDF3. In addition, translation is inhibited by demethylase FTO, promoted by methyltransferase METTL3/14, and upregulated under heat shock stress [37]. Intriguingly, m6A modification in circRNA occurs at low levels (approximately ~13% of total circRNAs had the m6A modification), and m6A-driven translation in some circRNAs is upregulated under heat shock stress [37]. These findings indicate that m6A-driven translation is commonly weak but can control circRNA translation with immediate and selective changes to respond to stress. Other mechanisms may be involved in translation without stress.
3. Biological roles of circRNAs in cancer

The ongoing efforts to elucidate circRNA functions have achieved fruitful results. In addition, mounting evidence shows that circRNAs are widely involved in the initiation and progression of human diseases, especially cancers, and could be a novel biomarker in cancer [23,24].

3.1. The Ying and Yang of circRNAs in cancer

CircRNAs are widely dysregulated via microarray or RNA sequencing and potentially play suppressive or oncogenic roles in multiple cancers [23,24]. Their relationship between suppressive and oncogenic circRNAs is similar to the ‘Yin’ and ‘Yang’ formed from the philosophy in ancient China (Fig. 3). CircRNAs narrate a beautiful circular molecular ‘tale’ about the balance of Yin and Yang via multiple routes in cells. If the balance of Yin and Yang is dysregulated, disease occurs.

As previously mentioned, circRNA levels are globally reducted in colorectal cancer (CRC) and are negatively correlated with proliferation [12]. In addition, circITCH is generally downregulated in esophageal squamous cell carcinoma, and overexpression of circITCH suppresses tumor growth via miR-7, miR-17 and miR-124 binding [70]. Consistent two early studies, other studies support the suppressive roles of some circRNAs in cancer. For example, circFOX3 is generally downregulated in breast tumor tissues and could suppress tumor growth and cancer cell viability [44,71]. Specifically, CircFOX3 serves as a miRNA sponge to buffer miRNA-mediated reductions in Foxo3 mRNA [44], a scaffold to promote the interaction between p21 and CDK2 that inhibits cell cycle progression [56], and a decoy to enhance the interaction between MDM2 and p53 that reduces MDM2-mediated ubiquitination of FOXO3 in breast cancer [71]. In parallel, low circHIAT1 expression is noted in clear cell renal cell carcinoma (ccRCC) and could mediate miR-195-5p/29a-3p/29c-3p-CDC42 signals to regulate ccRCC cell migration and invasion [72]. The expression of circZKSCAN1 was lower in hepatocellular carcinoma (HCC) and could regulate HCC progression via several cancer-related signaling pathways [73]. Cao’s group identified that circMT01 is significantly downregulated in HCC and can suppress HCC progression by acting as a miR-9 sponge [51]. Recently, Li et al. found that circHIPK3 is significantly down-regulated in bladder cancer tissues and cell lines. Over-expression of circHIPK3 effectively inhibited migration, invasion, and angiogenesis of bladder cancer cells, via sponging miR-558 to suppress heparanase expression [46]. Importantly, Zhang et al. discovered circFBXW7 driven by IRES encodes a novel 21-KDa FBXW7-185aa protein, and both of them are reduced in glioblastoma. Moreover, FBXW7-185aa can competitively bind USP28 to antagonizing USP28-induced c-Myc stabilization, thus suppressing glioma cell proliferation and cell cycle [74]. These studies have greatly enriched the suppressive roles of circRNAs and demonstrate how the loss of suppressive circRNAs is involved in multiple cancer types.

By sharp contrast, A large number of circRNAs are regulated during the epithelial to mesenchymal transition (EMT), suggesting that some circRNAs play specific biological roles in the EMT [33]. Given that the EMT is important for tumor invasion and metastasis, understanding the role of circRNAs in the EMT may provide new pathways for therapeutic intervention in cancer. In addition, Huang’s group found that silencing of circHIPK3 significantly inhibited cell growth in Huh7, HCT-116 and HeLa cell line, mechanistically by binding to miR-124 [45]. CircPVT1 is often upregulated in gastric cancer and promotes cell proliferation by sponging members of the miR-125 family [75]. These studies suggest the presence of oncogenic circRNAs. In CRC, the circCCDC66 and circS-7 are upregulated. Knockdown of these circRNAs inhibited tumor growth and cancer invasion via targeting different miRNAs [48,76]. In oral cancer, circRNA_100290 is upregulated, and knockdown of circRNA_100290 suppressed oral cancer cells proliferation in vitro and in vivo [49]. In glioma, circTBBK2 is upregulated and promotes glioma malignancy by targeting miR-217 [77]. Moreover, circMYLK is upregulated in bladder cancer and promotes bladder cancer progression through binding to miR-29a [78]. Interestingly, these findings demonstrate that circRNAs are involved in cancer mainly via sponging cancer-related miRNAs. However, whether these circRNAs play a role in cancer via other pathways remains unclear. Notably, Yang and others recently reported that circAmot1 is highly expressed in breast cancer and promoted tumorigenesis via interacting with c-myc to regulate its function and translocation [79]. Given that numerous oncogenic or suppressive mRNA or proteins are associated with cancer progression, it will be interesting to investigate whether other circRNAs are involved in cancer via interacting with cancer-related proteins or binding miRNA to maintain its stabilization or promote its degradation.

Remarkably, chromosomal translocations are often associated with the multiple tumor types including hematological and solid tumors [80]. Gene fusion is a product of chromosomal translocations and a common cause of malignancy. Interestingly, the cancer-associated rearranged genome could give rise to fusion circRNAs (f-circRNA) [81]. Moreover, f-circRNAs could promote transformation and cell survival. F-circM9s play critical roles in maintaining leukemic cells viability [81]. However, the specific mechanism of f-circRNA in cancer remains unclear. In addition, it is...
enticing to hypothesize that chromosomal translocations affect noncoding RNAs, such as fusion-IncRNA. Taken together, these findings revealed that the dysregulated circRNAs in cancer often exert their regulatory functions via different pathways (Table 1). A delicate balance between tumor suppressive circRNAs and oncogenic circRNAs is essential for cellular homeostasis. When imbalanced, tumor suppressive circRNAs may impair the protective effect on the cancer, whereas oncogenic circRNAs may promote cancer progression.

### 3.2. CircRNA is a promising biomarker with clinical implications

The existence of a huge number of circRNAs that may play roles in the progression of cancer phenotypes has important clinical implication. In addition, circRNAs are generally expressed in a tissue- and cell-type-specific manner and could help distinguish different tumors or predict treatment responses. Given that circRNAs are stable and resistant to RNA exonuclease or RNase R [25], circRNA exhibits the potential to serve as a biomarker of tumor diagnosis and therapy. As previously mentioned, from the tissue perspective, the CRC patients with high circCCDC66 or ciRS-7 expression correlated with a poor prognosis [48,76]. Moreover, the circCCDC66 could also be used to be a diagnostic marker for CRC [48]. In HCC, the decrease of circMTO1 may work as a poor prognosis predictor [51]. The ideal biomarkers should be relatively stable and easy to detect in body fluids. Intriguingly, recent studies reported that some circRNAs could be detected in human cell-free saliva or plasma by next-generation sequencing [82,83], paving ways for further cancer biomarker discoveries in body fluids. However, only a few circRNAs were identified, limiting any further analysis. Strikingly, approximately 2400 circRNAs were identified in human peripheral whole blood [84]. Moreover, circRNAs are enriched and stable in exosomes [13,85]. These exosomes that contain tumor-specific circRNAs may be largely released by tumor cells, protecting circRNAs from the RNases present in extracellular fluids. Additionally, tumor-derived exosomal circRNA could enter the circulation and measured for cancer detection. Serum exosomal circRNAs may distinguish patients with colon cancer from healthy individuals [13], suggesting that exosomal circRNAs may serve as promising cancer biomarkers. Furthermore, three circRNAs,

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Cancer phenotype</th>
<th>Cancer association</th>
<th>Clinical significance</th>
<th>Mechanism</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>circFoxo3</td>
<td>Inhibits tumor growth, cancer cell proliferation and survival</td>
<td>Downregulated in breast cancer</td>
<td>Not available</td>
<td>Binds to eight miRNAs; Forms circ-Foxo3-p21-CDK2 ternary complex and blocks the function of p21 and CDK2; Promotes MDM2-induced p53 ubiquitination and protects Foxo3 from MDM2-induced ubiquitination</td>
<td>[44,56,71]</td>
</tr>
<tr>
<td>circMTO1</td>
<td>Suppresses cell proliferation and invasion</td>
<td>Downregulated in HCC</td>
<td>Low circMTO1 had a poor prognosis</td>
<td>Mediates several cancer-related signaling pathways</td>
<td>[51]</td>
</tr>
<tr>
<td>circZKSCAN1</td>
<td>Inhibits cell proliferation, migration, and invasion</td>
<td>Downregulated in HCC</td>
<td>Serves as a potential diagnostic marker</td>
<td>Serves as a potential diagnostic marker</td>
<td>[73]</td>
</tr>
<tr>
<td>circTCH</td>
<td>Inhibits cell proliferation</td>
<td>Downregulated in ESCC</td>
<td>Not available</td>
<td>Serves as a potential diagnostic marker</td>
<td>[70]</td>
</tr>
<tr>
<td>circHAAT1</td>
<td>Inhibits cell migration and invasion</td>
<td>Downregulated in ccRCC</td>
<td>Low circHAAT1 predicted a poor prognosis</td>
<td>Serves as a potential diagnostic marker</td>
<td>[72]</td>
</tr>
<tr>
<td>circHIPK3</td>
<td>Inhibits cell migration, invasion, and angiogenesis</td>
<td>Downregulated in bladder cancer</td>
<td>Not available</td>
<td>Serves as a potential diagnostic marker</td>
<td>[46]</td>
</tr>
<tr>
<td>circFBXW7</td>
<td>Inhibits cell proliferation and cell cycle acceleration</td>
<td>Downregulated in glioblastoma</td>
<td>Low circFBXW7 predicted a poor prognosis</td>
<td>Encodes a FBXW7-185aa protein to competitively bind USP28 and “fixed” FBXW7x to degrade c-Myc</td>
<td>[74]</td>
</tr>
<tr>
<td>circCCDC66</td>
<td>Promotes cancer cell proliferation, migration, and metastasis</td>
<td>Overexpressed in CRC</td>
<td>High circCCDC66 predicted a poor prognosis</td>
<td>Functions as miR-31b, miR-93 and miR185 sponge for a subset of oncogenes.</td>
<td>[48]</td>
</tr>
<tr>
<td>circ-7</td>
<td>Promotes cell proliferation, migration, and invasion</td>
<td>Upregulated in CRC</td>
<td>High circ-7 correlated with poor prognosis</td>
<td>Targets miR-7 to regulate its targets</td>
<td>[76]</td>
</tr>
<tr>
<td>circRNA_100290</td>
<td>Promotes cell proliferation</td>
<td>Upregulated in OSCC</td>
<td>Not available</td>
<td>Serves as a potential diagnostic marker</td>
<td>[49]</td>
</tr>
<tr>
<td>circHIPK3</td>
<td>Promotes cell proliferation (Huh7, HCT-116 and HeLa)</td>
<td>Upregulated in HCC</td>
<td>Not available</td>
<td>Binds to miR-124 and inhibits miR-124 activity</td>
<td>[45]</td>
</tr>
<tr>
<td>circPVT1</td>
<td>Promotes cell proliferation</td>
<td>Upregulated in gastric cancer</td>
<td>Low circPVT1 correlated with poor prognosis</td>
<td>Serves as a potential diagnostic marker</td>
<td>[75]</td>
</tr>
<tr>
<td>circTTBK2</td>
<td>Promotes cell proliferation, migration, and invasion</td>
<td>Upregulated in glioma</td>
<td>CircTTBK2 positively correlated with the pathological grades of glioma</td>
<td>Serves as a potential diagnostic marker</td>
<td>[77]</td>
</tr>
<tr>
<td>circMYLK</td>
<td>Accelerates cell proliferation, invasion and angiogenesis</td>
<td>Upregulated in bladder cancer</td>
<td>High circMYLK correlated with low survival rates.</td>
<td>Serves as a potential diagnostic marker</td>
<td>[78]</td>
</tr>
<tr>
<td>circAmot1</td>
<td>Promotes cell proliferation</td>
<td>Upregulated in breast cancer</td>
<td>Not available</td>
<td>Serves as a potential diagnostic marker</td>
<td>[79]</td>
</tr>
<tr>
<td>f-circM9s</td>
<td>Contributes to cellular transformation, cell viability and resistance upon therapy</td>
<td>Not available</td>
<td>Not available</td>
<td>Serves as a potential diagnostic marker</td>
<td>[81]</td>
</tr>
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</table>

Hepatocellular carcinoma (HCC); Esophageal squamous cell carcinoma (ESCC); Colorectal cancer (CRC); Oral squamous cell carcinomas (OSCC); Clear cell renal cell carcinoma (ccRCC).
including circN4BP2L2, circGSE1 and circSMARCA5, were mainly present in serum-depleted exosomes compared with exosome-depleted serum [13]. It would be interesting to test whether circulating circRNAs are mostly located in the exosome but not in exosome-depleted serum or plasma. Moreover, the mechanism by which circRNAs enter the exosome and the roles of exosomal circRNAs remain unclear. In addition, whether exosomal circRNAs are involved in cell-to-cell communication remains an open question.

4. Challenges and future perspectives

CircRNAs were previously considered as a type of dark matter of life. Recently, with the development of RNA-seq and bioinformatic approaches, circRNAs have attracted widespread attention, and the mystery of circRNAs has gradually been unveiled. Some tools, such as find-circ [3], CIRI [86], MapSplice [16], CIRCexplorer [19], circRNA finder [87], and Acfs [88], have been developed to annotate circRNAs, and large amounts of circRNAs are stable and conserved and exhibit cell/tissue-specific expression. However, considering that the consistency of these tools remains relatively low [89], these computational tools are recommended to be used in combination to improve the sensitivity and specificity of identification.

Although circRNAs are primarily generated from protein coding genes and no endogenous circRNAs were associated with ribosomes, circRNAs were recently recognized as a pervasive class of noncoding RNAs in eukaryotic cells [27–30]. Therefore, we might easily ignore the coding potential of some circRNAs. However, we may first evaluate the ability of circRNAs to encode polypeptides or proteins. Accumulating evidence have presented multiple prediction pipelines supporting circRNAs translation via a cap-independent manner [35–37]: (1) the existence of an ORF with sufficient length; (2) an ORF across the backsplice site; (3) a regulatory element located upstream of the ORF, such as IRES, a specific sequence exhibiting IRES-like activity, a translation initiation site (TIS) or m^5^A motifs (RRACH fragment (R = G or A; H = A, C or U)). These pipelines provide an important framework moving forward. It is worth looking forward to developing a coding potential prediction software for circRNAs, which is similar to CPAT [90]. To date, little information is available regarding the functions of proteins derived from circRNAs [74], so it is a topic worthy of exploring.

In addition to the functions described above, such as RNA-RNA and RNA-protein interactions, we cannot exclude the possibility that some circRNAs located in the nucleus could interact with DNA to regulate transcription or replication. To study circRNA function, overexpression or knockdown approaches are designed to specifically manipulate circRNA expression. Reverse complementary sequences are often incorporated into the vector to facilitate circRNA overexpression. Additionally, siRNA are designed to specifically target the backsplicing junction. CRISPR/Cas9 genome editing disrupts one or both of the flanking intronic repeats of the nearby circular RNA without affecting linear RNA. Furthermore, some online databases provide a good resource for circRNA functional research, such as Circ2Traits [91], CircInteractome [63], CSCD [92] and exoRbase [93]. Strikingly, Piwecka et al. firstly discovered an in vivo loss-of-function circRNA phenotype using CRISPR/Cas9 via micro-injection of one-cell embryos [52]. Given that circHIPK3 is highly conserved [47] and abundant, it will be interesting to identify circHIPK3 phenotype in vivo. Given that the functions of most circRNAs remain unknown and that recent studies have elucidated the functional roles of a handful of circRNAs in multiple biological processes [27], there is still a long way to go. However, these methods would help in the assessment of whether a new circRNA is functional with a single or multiple functions similar to circFoxo3.

To date, some circRNAs have been reported to play essential roles in multiple cancers [24], providing novel insight into cancer research. However, current research on the roles of circRNAs in cancer remains in its infancy. With the application of high-throughput screening technology, more dysregulated circRNAs in cancer will be discovered. Whether and how the dysregulated circRNAs play roles in cancer would be interesting to explore. Considering that SNPs are typically associated with the risk and progression of cancer [94], it is worthwhile to investigate whether SNPs are present in circRNA, how SNPs in circRNAs are involved in cancer, and the clinical significance of SNPs in circRNAs. Interestingly, a website is available that evaluates the association of circRNAs with the GWAS-associated SNPs (http://gyanxet-beta.com/circdb/) [91]. As previously described [81], f-circRNA may be responsible for some cancers. However, whether f-circRNA could be exported to the extracellular area (blood or exosome) and be applied to the diagnosis of cancer is an important question to be addressed. Moreover, considering the urgent need for clinical disease detection using non-invasive biomarkers, circRNAs could serve as ideal candidates as cancer biomarkers, given their stability and specific expression. A better understanding of the roles of circRNAs in cancer will help us develop circRNA-based diagnostic tools and therapies for cancer. For instance, if properly modified and packaged, circRNAs would be delivered to the cell to affect the cell fate, acting as a regulatory element. Nevertheless, this field is still in its infancy, and considerable research is needed to incorporate circRNA into clinical practice.

CircRNAs are becoming a new research frontier. With the increase in studies of circRNAs, an increasing number of circRNAs will be discovered and characterized in many species. There is an urgent need to develop a standard naming system given that name standardization would help apply bioinformatic and biochemical research to circRNA research. Although we have made some progress in the field of circRNA [27], other important aspects are not yet fully understood. For example, how are circRNAs transported within the cell? Where are circRNAs distributed in the cell? What sequence and structural elements in circRNA facilitate their cellular functions? How are circRNAs degraded in cells? Answering these questions will help unveil new insight into circRNA biology. Revealing information regarding circRNAs would expand our understanding of the complex world of eukaryotic transcriptomes. As an emerging key player in the RNA world, circRNAs may widely affect life processes, serve as diagnostic or predictive biomarkers of disease and provide a new path for the treatment of diseases.

Conflicts of interest

The authors declare that they have no conflict of interest.

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