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REVIEW

Functional role of circular RNAs in cancer development and progression

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ABSTRACT

Circular RNAs (circRNAs) are a large class of endogenously expressed non-coding RNAs formed by covalently closed loops through back-splicing. High throughput sequencing technologies have identified thousands of circRNAs with high sequence conservation and cell type specific expression in eukaryotes. CircRNAs play multiple important roles in cellular physiology functioning as miRNA sponges, transcriptional regulators, RBP binding molecules, templates for protein translation, and immune regulators. In a clinical context, circRNAs expression is correlated with patient's clinico-pathological features in cancers including breast, liver, gastric, colorectal, and lung cancer. Additionally, distinct properties of circRNAs, such as high stability, exonuclease resistance, and existence in body fluids, show promising role for circRNAs as molecular biomarkers for tumor diagnosis, non-invasive monitoring, prognosis, and therapeutic intervention. Therefore, it is critical to further understand the molecular mechanism underlying circRNAs interaction in tumors and the recent progress of this RNA species in cancer development. In this review, we provide a detailed description of biological functions, molecular role of circRNAs in different cancers, and its potential role as biomarkers in a clinical context.

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1. Background: origin of circRNAs as functional non-coding RNAs

Circular RNAs (circRNAs) are a large group of non-coding RNAs formed by back-splicing. Initial microscopy studies provided the early evidence of circRNAs under denaturing conditions primarily in viral genetic materials [1–3]. Following the observation of circular molecules in viral genome, the first circRNAs in eukaryotic cells were confirmed in the cytoplasmic region of HeLa cells [4], and yeast mitochondrial RNA [5]. Subsequent evidence on individual circRNAs was then continually reported in different human and rat tissues, for example circRNAs derived from *ETS-1, SRY, P450, ABP, DYSTROPHIN, MBL*, and *AML* [6–12]. Though multiple experiments prove the existence of circRNAs, the identified circRNAs were generally less abundant than the linear products from the same parental genes. Therefore, circRNAs were considered as rare events with unclear biological functions before the advent of genome-wide sequencing technologies. The development of high throughput sequencing has enabled in-depth characterization of circRNAs for identification, abundance, and potential functions. Among the major features of high throughput sequencing includes longer read lengths, improved circRNA mining bioinformatics algorithms, and more importantly, ribosomal RNA (rRNA)-depleted non-polyadenylated RNA sequencing [13–15]. Since then, the focus of circRNA research has shifted to elucidate the biogenesis and the functional roles of circRNAs. Several biogenesis models have been proposed, including direct back-splicing with ALU and inverted repeats complementation [14,16,17], exon lariat [18], and RNA binding protein (ADAR, MBL, DHX9, FUS, RBM20, and QKI) mediated models [16,19–23]. Furthermore, several biological functions such as microRNA (miRNA) sponges, transcriptional regulator, protein interaction, protein translation, regulator of cancer progression, and immune responses have been implicated (Figure 1). Remarkably, cancer development and progression has been shown to be associated with deregulated circRNA expression. Regulation of gene expression by circRNAs through sponging disease-related miRNAs, forming a complex network of miRNA-circRNA-ncRNA and circRNA-protein interaction will be further discussed in this review. As a result of these diverse functions, aberrant expression of circRNAs impacts the development of a wide array of human diseases, particularly cancer. Furthermore, the distinct characteristics of circRNAs, including long half-life, resistance to exonuclease, expression in tissue, serum, urine, blood, and saliva, make it a promising biomarker with tremendous potentials in a clinical setting. In this review, we have summarised the key functions of circRNAs in the specific context of cancer development and progression and its putative biomarker potential.

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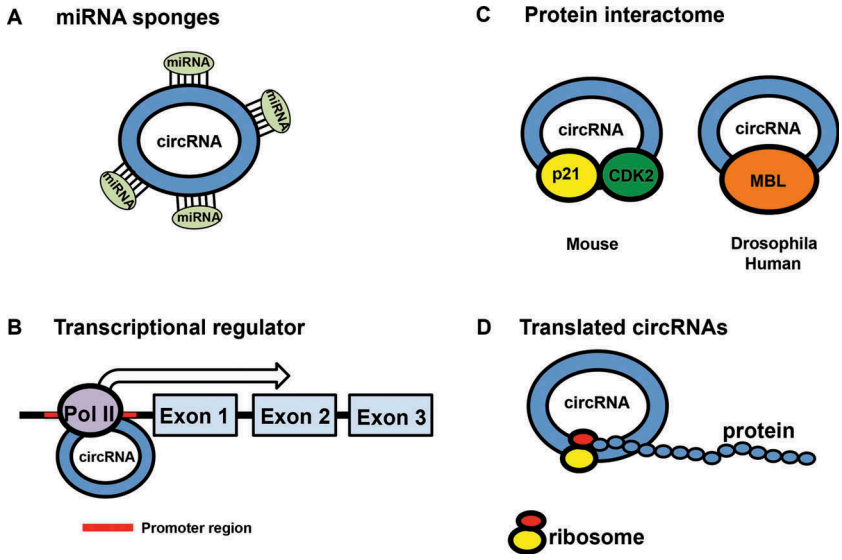

Figure 1. Key functions of circRNAs. (A) CircRNA serves as miRNA sponge that harbors multiple miRNA binding sites that indirectly controls gene expression; (B) CircRNA functions as a transcriptional regulator via binding to RNA polymerase II; (C) CircRNAs act as a protein interactome. In human and *Drosophila*, RBP (MBL) binds to circRNA to compete with linear alternative splicing; In mouse, cell cycle related proteins bind to circRNAs to strengthen p21/CDK2 interaction and block cell cycle progression; (D) CircRNA that contains open reading frame (ORF) and in-frame stop codon is translated into proteins in a splicing-dependent, cap-independent manner.

2. Known molecular mechanism of circular RNAs

2.1. Microrna sponge

Growing evidence for the functional roles of circRNAs has been discovered in different cellular physiologies (Figure 1). The mechanism of action of circRNAs that received the most attention is their miRNA sponging activities (Figure 1(A)). CircRNAs sequester miRNAs via complementary RNA base-pairing and thus prevent miRNAs from binding their mRNA targets. The first line of evidence for miRNA sponging activity is proven both *in vitro* and *in vivo* in CDR1-as circRNAs [15,24]. Both experiments show that CDR1-as is densely bound by AGO and harbor seed regions for miR-7. *In vitro* assay shows that CDR1-as binds to miR-7 [24]. In addition, over-expression of CDR1-as reduces the miR-7 levels, which then enhances the expression of miR-7 target genes. Using zebrafish and mouse as model systems, *in vivo* analysis demonstrates the role of CDR1-as in brain development through regulating the activity of miR-7 [15]. Furthermore, the outcome of over-expression of CDR1-as mimics the phenotypes of miR-7 knockdown with morpholinos, which causes the reduction of mid-brain size in zebrafish embryo [15]. In addition, miR-671 has also been proposed to directly cleave CDR1-as due to its almost perfect match, suggesting additional functions of CDR1-as beside miR-7 inhibition [25,26]. In mouse, CDR1-as KO mouse showed impaired sensorimotor gating [25]. The loss of CDR1-as demonstrated a critical interaction between circRNA and miRNA in brain development of zebrafish embryo and brain function of mouse through affecting miR-7 and miR-671 expression [25]. Following the description of circRNAs as miRNA sponges, numerous circRNAs have been implicated to bind disease-associated miRNAs, suggesting the involvement of circRNAs in disease development as discussed in later sections. Nonetheless, genome-wide sequencing analysis reveals that miRNA sponging activity cannot be widely applied across all circRNAs [14,15,27,28] since very few of circRNAs harbor more than 10 seed regions for a single miRNA [28].

2.2. Transcriptional regulators

CircRNAs function as transcriptional regulators (Figure 1(B)). Additionally, nuclear residing exon-intron circRNAs (ElciRNAs), associate with RNA polymerase II, interact with U1 snRNP and Pol II transcription complex at the promoter of their parental genes, thereby control the expression of parental genes [29]. Taken together, circRNAs act as transcriptional regulators to control host gene expression.

2.3. Protein interactome

Multiple evidence demonstrated the role of non-coding RNA in control of gene expression both at the transcription and post-transcriptional levels via physical interaction with RNA binding proteins or other non-coding RNAs [30]. It raised the possibility that circRNAs might have similar roles in mediating cellular functions through serving as a platform for protein interaction (Figure 1(C)). For instance, circRNAs are associated with transcriptional related proteins AGO2 and RNA Pol II [29]. In addition, circ-Mbl competes for binding to splicing related protein Mbl [19]. Furthermore, circFoxo3 forms a ternary complex with cell cycle related proteins, such as CDK2 and p21 [31]. Besides, a subfamily of circRNA is also associated with IMP3, a known oncofetal and tumor marker with post-transcriptional regulation roles [32]. Furthermore, circPABPN1 binds to HuR, and thus

Table 1. CircRNA and protein interacting partners.

CircRNAs	Protein involved	Functions	References
circEIF3J, circPAIP2	RNA Pol II, U1	Regulate parental host gene expression	Li et al. [29]
circMbl	MBL	Competes for alternative splicing with Mbl transcript	Ashwal-Fluss et al. [19]
circFoxo3	CDK2, p21	Blocks cell cycle progression	Du et al. [31]
circNFATC3, circANKRD17	IMP3	Subclass of RNA binding protein potentially linked to circRNA biogenesis	Schneider et al. [32]
circPABPN1	HuR	Binds to HuR RBP, serves as a competition between circRNA and cognate mRNA, affecting PABPN1 protein translation	Abdelmohsen et al. [33]

prevents HuR from binding to PABPN1 mRNA, thereby reduces PABPN1 translation [33]. The biological functions of related circRNAs and protein interaction are listed in Table 1.

2.4. Translation of circRNAs

In vitro introduction of internal ribosome entry site (IRES) and reading frames result in translation of engineered circRNAs [34–37]. Therefore, it is possible that AUG-containing exon within circRNAs could be translated into mini proteins (Figure 1(D)). Guo et al showed that majority of the circRNAs provides no evidence for translation [28]. However, the first evidence of protein coding circRNA in eukaryotes is described for circ-ZNF609 [38]. This circRNA contains a start codon with an in-frame stop codon created upon circularization. Translated circ-ZNF609 controls myoblast proliferation, associates with heavy polysomes, and is translated through splicing-dependent, cap-independent mechanism [38]. In addition, a number of circRNAs, for example, circMBL, circDMD, and circFMN, behave as 'mRNA trap' which binds to cognate linear mRNA and sequesters the mRNA from undergoing translation, ultimately leading to reduced protein expression level [10,19,39].

3. Additional roles of circRNAs

3.1. Cancer association and potential as a biomarker

The expression of circRNAs is associated with several cancers, which will be reviewed in the following sections (Figure 2(A)). There are two major experimental readouts in measuring the expression of circRNAs in cancer tissues or cell lines: global expression changes (circRNA microarray or RNA-sequencing) and specific circRNA expression measurement.

3.2. Immune responses

CircRNAs have been implicated in mediating immune responses (Figure 2(B)). For example, circRasGEF1B positively regulates the expression of TLR4/LPS- induced *ICAM-1* mature mRNA stability, thereby controlling the innate immune responses [40]. Furthermore, cellular mechanism recognises foreign circRNAs through intron identity, which enables the sensing of self and

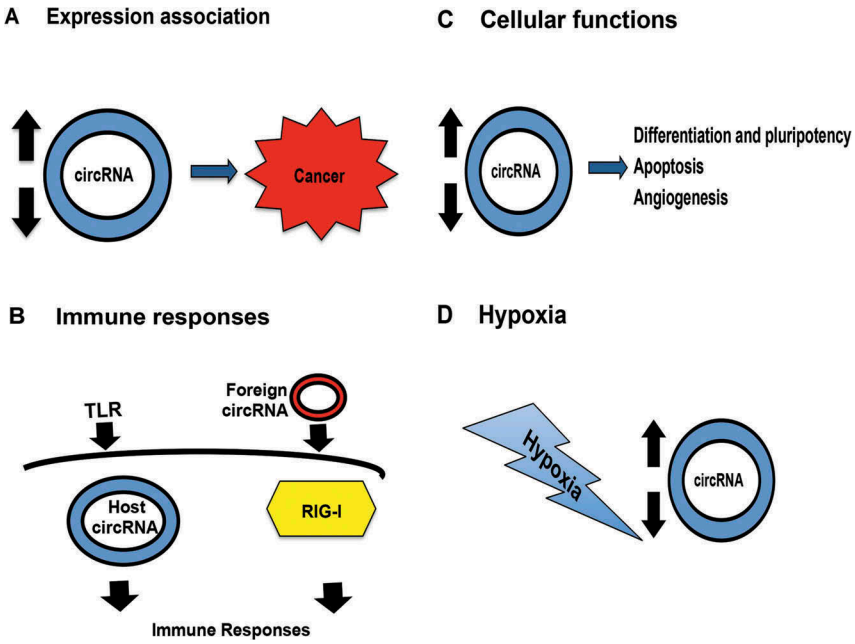

Figure 2. Roles of circRNAs. (A) Aberrant circRNAs expression associates with different cancer outcome. (B) Immune signaling, such as Toll-like receptor, induces the production of host circRNAs (left). Foreign circRNAs evoke RIG-I mediated immune responses via intron identity (right). (C) CircRNAs expression associates with cellular functions. (D) Hypoxia induces circRNAs expression.

foreign circRNAs within the cytoplasm of the host [41]. Intriguingly, the mechanism of the lack of 5′ triphosphate group in circRNAs in activating the RIG-I pathway remains unknown.

3.3. Cellular differentiation and pluripotency

CircRNAs also play additional roles in cellular functions (Figure 2(C)). From a developmental context, circRNAs are found to be abundantly expressed and upregulated in epidermal stem cell differentiation and largely independent of host gene expression. In particular, circHECTD1 and circZNF91 are predicted to have multiple miRNA and AGO2 binding sites. For example, circZNF91 harbors 24 sites for miR-23b-3p, a key player in keratinocyte differentiation [42]. In addition, Yu et al. showed the involvement of circRNAs in embryonic stem cells for maintaining pluripotency. They showed that circBIRC6 (AGO2-associated) and circCORO1C (non AGO2-associated) act as regulators in pluripotent state. However, only circBIRC6 acts as a 'miRNA sponge' to miR-34a and miR-145 to modulate human pluripotency and differentiation. Its biogenesis is promoted by ESRP1 splicing factor, whose expression is controlled by OCT4 and NANOG [43].

3.4. Apoptosis

Several circRNAs are involved in regulating apoptosis. Overexpression of circFoxo3 releases Foxo3 from MDM2-dependent degradation and leads to Puma and Bax-mediated apoptosis [44]. In contrast, unlike circFoxo3, circUBAP2 is shown to inhibit apoptosis [45].

3.5. Angiogenesis

CircFoxo3 has been shown in the inhibition of angiogenesis [46], while hypoxia- induced circZNF292 shows proangiogenic activities [47]. Additionally, circMYLK promotes angiogenesis via VEGFA/VEGFR2 pathway [48].

3.6. Hypoxia

Hypoxia levels positively correlate with the prognosis of cancer patients and serves as a key feature of most solid tumors [49]. Cancer cells increase HIF1α transcription factor expression and promote angiogenesis, proliferation, and metastasis [50]. After hypoxia induction, several hypoxia-associated circRNAs were induced in endothelial cells including circZNF292, circAFF1, circTHSD1, circDENND4C, circSRSF4, and circFOXJ3 [47], (Figure 2(D,)). Further experiment on silencing HIF1α in breast cancer cell line MDA-MB-231 showed that hypoxia-induced circDENND4C is a HIF1α associated circRNA. CircDENND4C mediates breast cancer cells proliferation in hypoxic environment and the expression of this circRNA positively correlates with HIF1α level and the tumor size in 30 paired breast cancer and adjacent normal tissues [51].

3.7. Growing number of databases of circRNAs

Over thousands of circRNAs have been identified and documented by different research groups. Hence, the up to date compilation of circRNAs databases and resources is necessary for better navigation and experimental design. Existing databases include circBase [52], circInteractome [53], CircNet [54], circPedia [55], circRNABase [56], circRNADb [57], Circ2Traits [58], CSCD [59], deepBase v2.0 [60], plant-specific PlantcircBase [61] and SomamiR v2.0 [62],(Table 2). In particular, CSCD [59] serves as a comprehensive database for cancer-specific circRNAs navigation.

4. Role of circular RNA in cancer progression

4.1. Aberrant expression of circRNAs in cancer

CircRNA expression has been confirmed in various human cell types [15,63], where they play important physiological roles such as cell proliferation and hematopoiesis [64,65]. Deregulated expression of circRNAs and its clinical significance has been reported in several cancers. Global circular RNA abundance negatively correlates with cellular proliferation and a global reduction of circular RNA abundance has been reported in colorectal cell lines, ovarian cancer and idiopathic lung fibrosis compared to normal human tissues [64]. Besides, a genome-wide circRNA array study in breast cancer detected 1155 differentially expressed circRNAs (715 up- and 440 down- regulated) in breast cancer tissues [66]. In blood cancer, circRNA microarray identified 464 differentially expressed circRNAs (147 up and 317 down- regulated) in CN-AML patients compared to healthy controls, in which 12 dysregulated circRNAs were expressed 10 fold more than healthy controls [67]. In bladder carcinoma, two independent microarray studies detected 469 (285 up- and 184 down-regulated) [68], and 571 (47 up- and 524 down- regulated) dysregulated circular transcripts respectively [69]. In pancreatic ductal adenocarcinoma (PDAC), using circRNA microarray, a total of 351 (209 up- and 142 down- regulated) circRNAs were aberrantly expressed between 6 PDAC cancer samples and paired adjacent tissues [70]. Interestingly, more circRNAs were upregulated rather than downregulated in liver cancer. A microarray study identified 127 (113 up- and 14 down- regulated) differentially expressed circRNAs in liver cancer while another independent study reported a total of 226 (189 up- and 37- down regulated) differentially expressed circRNAs [71,72].

The expression levels of specific circRNAs such as hsa_circRNA_103809, hsa_circRNA_104700 and hsa_-circ_001988 are downregulated in cancerous tissue compared to the normal adjacent tissue [73,74]. This is significantly associated with clinicopathological features in colorectal cancer (CRC) patients such as metastasis, differentiation and perineural invasion respectively [73,74]. On the other hand, circ_BANP and hsa_-circ_0000069, and hsa_circ_001569 have been shown to be over-expressed in CRC cancerous tissue and proposed to be a prognostic and therapeutic marker because of their role in cancer progression [75–77]. In addition, a microarray analysis was performed by one group to study chemoradiation resistance (CRR) in response to 5-fluorouracil in CRC patients [78]. This study had

Table 2. List of available up-to-date circRNA databases.

Database	Cell types	Highlights	Link
CircBase	*H. sapiens* (hg19) *M.musculus* (mm9) *C.elegans* (ce6) *L. chalumnae* (latCha1) *L. menadoensis* (latCha1) *D.melanogaster* (dm3)	One of the earliest and most comprehensive databases. The custom python scripts can be downloaded	http://www.circbase.org
circInteractome	109 datasets of RNA binding proteins (RBP) and circRNAs for RNA binding sites [39].	Searches RBP binding to a circRNA and sequences upstream or downstream of circRNAs Identifies RBPs binding to circRNA junctions Identifies miRNAs targeting circRNAs Designs divergent primers Designs siRNAs specific for circRNAs	https://circinteractome.nia.nih.gov
CircNet	464 RNA-seq samples without PolyA selection from 26 different human tissue samples	Provides a total of 212,950 circRNAs Provides a total of 34,000 circRNAs with junction sites > 3, as highly expressed circRNAs. Provides circRNA expression profiles across 26 different human tissues Predicts circRNA-miRNA interactions and regulatory networks Provides genomic annotation of circRNAs using integrated genome browser	http://circnet.mbc.nctu.edu.tw
CircPedia	31 human 26 mouse 30 fly 12 worm	Annotate alternative back-splicing in circRNAs across different cell lines with circRNA characterization pipeline CIRCexplorer2	http://www.picb.ac.cn/rnomics/circpedia/
circRNABase	*H. sapiens* (hg19) *M.musculus* (mm9)	Predicts miRNA-circRNA interactions by overlapping circRNA sequence with CLIP-seq peaks from miRNA targets	http://starbase.sysu.edu.cn/mirCircRNA.php
circRNADb	CD_19 CD_34 HEK293 Neutrophils Hs68 H9 hESCs Leukemia Leukocyte Normal brain tissue Glioblastoma Oligodendroma	Contains 32,914 exonic circRNAs with 16,328 protein-coding annotation A total of 46 circRNAs from 37 genes have corresponding proteins expressed based on mass-spectrometry	http://reprod.njmu.edu.cn/circrnadb
Circ2Traits	Data sources taken from 1953 predicted human circRNAs [15], and miR2Disease (174 different human disease) [49]	Measures the likelihood of a circRNA and disease association using hypergeomtric test, $p < 0.01$ Visualizes circRNA-miRNA-mRNA-lncRNA interactome network for individual disease Information about disease associated SNP in circRNA loci	http://gyanxet-beta.com/circdb/
CSCD	Human cancer 228 total RNA or polyA(-) RNA seq samples from cancer and normal cell lines	Identifies 272,152 cancer-specific circRNA Predicts miRNA response element sites Predicts ORF for translatable circRNAs Predicts RBP sites of each circRNA	http://gb.whu.edu.cn/CSCD
deepBase v2.0	*H. sapiens* *M.musculus* *Drosophila* *C. elegans*	Predicts 101,739 circRNAs Provides a distribution of circRNAs in each species: Caenorhabditis (0.5%), Fly (5.6%), Mouse (1.6%), Human (92.3%)	http://biocenter.sysu.edu.cn/deepBase/
PlantcircBase	*Arabidopsis thalina* (TAIR10) *Hordeum vulgare* (ASM32608v1.26) *Oryza sativa* (RGAPv7) *Solanum lycopersicum* (SL2.40.25) *Zea mays* (AGPv3.22)	Predicts circRNAs as miRNA sponges. Provides plant circRNAs with related information (sequence, host genes, expression, experimental validation)	http://ibi.zju.edu.cn/plantcircbase/
SomaMIR v2.0	*H. sapiens* (Contains 620 types of cancer)	Predicts somatic mutations in miRNA target sites and displays somatic mutations within 3′ UTR of genes, lncRNAs, and circRNAs which creates or disrupts the target sites	http://compbio.uthsc.edu/SomamiR

identified 71 circRNAs that are differentially expressed in chemoradiation-resistant CRC cells. Out of these expression of 5 circRNAs was validated with quantitative reverse transcription PCR (qRT-PCR) and interacts with 355 miRNAs according to bioinformatics predictions [78]. Several other circular RNAs have been shown to play a role in CRC progression by acting as miRNA sponges or through transcriptional regulation and have been reviewed elsewhere [79]. Additionally, downregulation of circRNA abundance at a global level has been shown in several colon cancer cell lines having a mutant *KRAS* indicating a role of circRNA in tumorigenesis [80].

In gastric cancer (GC), hsa_circRNA_002059, hsa_circ RNA_0000181 and hsa_circ_0000190 expression has been shown to be downregulated in cancerous tissue compared to adjacent normal tissue with significant correlation to metastasis [81–83]. Based on the stability of hsa_circRNA_0000181 and hsa_circ_0000190 expression in tissue and plasma samples, they had been proposed to be novel biomarkers for prognosis and

diagnosis of gastric cancer [82,83]. At the same time, another study reported that the expression of circPVT1 that was derived from PVT1 gene is upregulated in GC tissue and acts as a sponge for miR-125 family [84]. The levels of circPVT1 has been proposed to be an independent prognostic biomarker for disease-free and overall survival in GC patients [84]. Moreover, in a cohort of 51 patients, hsa_circRNA_0067934 was significantly overexpressed in esophageal squamous cell carcinoma (ESCC) compared to the adjacent normal tissue and was associated with poor cellular differentiation [85].

Additionally, by combining results from 5 gastric cancer (GC) cell lines and 257 tumor tissue specimens it was found that hsa_circRNA_0001895 expression levels were significantly correlated with patient's clinicopathological factors suggesting its crucial role in gastric cancerogenesis and therefore is a potential biomarker for disease prognosis prediction [86]. In a similar study, hsa_circRNA_0003159 was shown to be downregulated in GC tissue compared to the adjacent normal tissue which was of diagnostic significance [87]. Global circRNA expression profiling in GC found a list of top 10 upregulated and downregulated circRNAs that have a significant aberrant expression in GC, amongst which hsa_circ_ 0014717 was downregulated in 77.2% gastric cancer tissues [88].

In prostate cancer, circSMARCA5 is an androgen-induced circRNA. In a study by Kong et al, circSMARCA5 is upregulated in 5 prostate cancer cell lines and 21 paired prostate cancer tissue samples compared with match normal tissues [89].

In hepatocellular carcinoma (HCC), hsa_circ_0001649 expression was downregulated in 89 paired samples of HCC and adjacent tissues, and its expression was correlated with tumor size and tumor embolus [90]. In addition, it has also been shown that hsa_circ_0004018 expression is lower in HCC and correlates with serum alpha-fetoprotein (AFP) level tumor diameter, differentiation, and tumor-node-metastasis stage [91]. However, another observational study showed that hsa_circ_0005075 expression was associated solely with the tumor size but not with other clinicopathological characteristics [92]. Interestingly, a detailed study on both ZKSCAN1 mRNA and circZKSCAN1 showed that the expression level of both transcript species was downregulated in HCC [93]. Additionally, knockdown of both species accelerated cell proliferation, migration, and invasion, while over-expression repressed the progression of HCC [93].

4.2. Network of circRNA-miRNA-mRNA in cancer

Recently, a study on gastric cancer (GC) tissues identified a network of circRNA, miRNA and mRNAs that are differentially expressed between the GC tissues and adjacent normal tissue samples [94]. This raises a possibility of a direct linkage between the regulatory properties among the network of circRNA-miRNA-mRNA expressions.

For instance, a regulatory network of circ_0006528-miR7–5p-Raf1 has been proposed in breast cancer. There is a negative correlation between circ_0006528 and miR-7–5p expression in Adriamycin (ADM) resistant cancer tissues. Raf1 is a direct target of miR-7–5p [95]. Knocking down circ_0006528 in MCF-7/ADM and MDA-MB-231/ADM cell lines reduced Raf1 mRNA and protein levels, suggesting a regulatory role of circRNA-miRNA-mRNA axis in ADM resistant breast cancer [96].

In addition, a network of circHIAT1/miR-195-5p/29a-3p/29c-3p/CDC42 is involved in clear cell renal cell carcinoma (ccRCC) development [97]. Androgen receptor suppressed circHIAT1 expression resulting in deregulated miR-195-5p/29a-3p/29c-3p expression, ultimately leading to increased CDC42 expression to enhance ccRCC cell migration and invasion. CircHIAT1 possibly acts as a 'miRNA reservoir' to stabilise miRNAs in this context.

Moreover, a circRNA and miRNA expression study in hepatocellular carcinoma (HCC) demonstrated a potential regulatory role of circRNA_000839/miR-200b/RhoA in the pathogenesis of HCC. This study indicated an inverse correlation between miR-200b with RhoA and circ_000839, suggesting a role of this interplay for miR-200b in suppression of the invasion and migration in HCC [98].

Furthermore, a microarray expression study in bladder carcinoma demonstrated a differential expression profile of circTCF25/miR-103a-3p/miR-107/CDK6 regulatory pathway. Over-expression of circTCF25 promotes proliferation and migration of EJ and T24 bladder cancer cell lines [68].

4.3. Oncogenic properties of circRNAs

CircRNAs also have oncogenic and proto-oncogenic roles to promote cancer formation. Fusion circRNAs (f-circRNA) are formed as a result of cancer associated genomic translocations and have tumor-promoting properties such as increased cell viability, resistance to therapy and cellular transformation in leukemic cells *in vivo* [99]. For example, cZNF292 has been shown to promote tube formation in gliomas and its inhibition is associated with suppression of tube formation by inhibition of cell cycle progression [100]. In non-small cell lung cancer, over expression of circRNA_100876 in tumor tissues compared to adjacent non-tumorous tissue has been correlated to lymph node metastasis, tumor staging and overall survival which is of prognostic and therapeutic significance [101].

4.4. CircRNA and miRNA sponging activity in cancer

MiRNA and circRNA interaction have also been reported in multiple cancer types (Table 3). For example in bladder cancer, upregulation of circTCF25 has been shown to downregulate miR-103a-3p and miR-107 and at the same time increase CDK6 expression [68]. This interaction has been linked to increased proliferation and migration both *in vitro* and *in vivo*. Interestingly, analysis of differentially expressed mRNA, lncRNA and circRNA in bladder cancer revealed regulatory networks whose role in pathogenesis of bladder

Table 3. miRNAs sponge activity of circRNAs in cancer and clinical outcomes.

Cancer type	circRNA	miRNA	miRNA targets	Outcome	References
Bladder	circTCF25	miR-103a-3p, miR-107	CDK6	Increase proliferation and migration	Zhong et al. [68]
Bladder	circMYLK	miR-29a	VEGFA	Relieve the repression and activate Ras/ERK pathway	Zhong et al. [48]
Bladder	circHIPK3	miR-558	HPSE	Suppress HPSE expression	Li et al. [69]
Breast	circABCB10	miR-1271	-	Promote proliferation and progression	Liang et al. [103]
Breast	circFoxo3	miR-22 miR-136 miR-138 miR-149 miR-433 miR-762 miR-3614-5p miR-3622-5p	-	Inhibit tumor growth and angiogenesis	Yang et al. [46]
Colorectal	hsa_circ001569	miR-145	E2F5, BAG4, FMNL2	Proliferation and invasion	Xie et al. [77]
Esophageal	circITCH	miR-7 miR-17 miR-214	ITCH	Promote phosphorylated Dvl2, inhibit Wnt/b-catenin pathway	Li et al. [104]
Gastric	hsa_circLARP4	miR-424	LATS1	Inhibit proliferation	Zhang et al. [105]
Gastric	hsa_circ100269	miR-630	-	Proliferation	Zhang et al. [106]
Hepatocellular carcinoma	ciRS-7	miR-7	-	Microvascular invasion	Xu et al. [107]
Hepatocellular carcinoma	circRNA-100338	miR-141-3p	MTSS1 (in silico)	Promote metastatic progression	Huang et al. [72]
Hepatocellular carcinoma	circMTO1	miR-9	p21	Suppress tumor development, correlate with poor prognosis	Han et al. [109]
Liver	circFUT8	miR-570-3p miR-17-3p miR-552-3p			Ren et al. [71]
Liver	circZFR	miR-511-5p miR-130b-5p miR-642a-5p miR-532-3p mir-329-5p			
Liver	circIPO11	miR-659-3p miR-424-5p miR-106a-3p			

cancer has been demonstrated [102]. Besides, in bladder carcinoma, circMYLK sponges miR-29a and relieve the repression of *VEGFA*, leading to activation of VEGFA/VEGFR2 and downstream Ras/ERK pathway [48]. In addition, a circularised exon 2 from *HIPK3* gene, circHIPK3, binds to miR-558, and suppresses the HPSE expression. Over-expression of circHIPK3 inhibits migration and invasion *in vitro*, while suppresses the growth, metastasis, and angiogenesis of bladder cancer cells *in vivo* [69].

Additionally, circABCB10 is abnormally upregulated in breast cancer tissue. The sponge activity of circABCB10 on miR-1271 resulted in breast cancer proliferation and progression [103]. Additionally, a mechanistic study on all three forms of Foxo3; circFoxo3, *Foxo3* mRNA, and pseudogene (*Foxo3P*), showed that all three forms suppress tumor growth and cell proliferation, and circFoxo3, *Foxo3P*, and *Foxo3* are targets of miRNAs [46].

In colorectal cancer (CRC), *in vitro* over-expression of hsa_circ_001569 in SW480 and HCT116 cells, and silencing of hsa_circ_001569 in SW620 and LOVO cells showed that this circRNA is a positive regulator in mediating cell proliferation and invasion through sponging the miR-145 expression levels, thereby upregulating its targets such as *E2F5, BAG4*, and *FMNL2* [77].

In esophageal squamous cell carcinoma (ESCC), circITCH sponges multiple miRNAs (miR-7, miR-17, and miR-214), which subsequently increase the ITCH expression. Increased expression of ITCH promotes Dvl2 degradation, and thus inhibits Wnt/β-catenin pathway in ESCC [104].

In gastric cancer (GC), hsa_circRNA_LARP4 has been shown to sponge miR-424 level which inhibits tumor proliferation and regulates its target gene *LATS1* gene in GC [105]. Hsa_circRNA_LARP4 has therefore been proposed to be a novel tumor suppressor and a potential biomarker in GC [105]. Moreover, hsa_circRNA_100269 downregulates the expression of miR-630 and the expression of hsa_circRNA_100269 negatively correlated with that of miR-630 in GC tissues [106]. The interaction of hsa_circRNA_100269 and miR-630 has been shown to regulate tumor cell proliferation in GC [106].

In liver cancer, circular RNA regulates expression of miRNAs by acting as a miRNA sponge and have clinical consequences, especially in hepatocellular carcinoma (HCC) [107]. CiRS-7 acts as a miRNA sponge and inhibitor of miR-7, a tumor suppressor. CiRS-7 may be considered as a risk factor in HCC as it is significantly correlated with hepatic microvascular invasion [107,108]. Besides, another study reported that three circRNAs, namely, circFUT8, circZFR, and circIPO11 were upregulated in 40 clinical samples. However, the sponging activities of the predicted circFUT8-miR-570-3p/miR-17-3p/miR-552-3p, circZFR-miR-511-5p/miR-130b-5p/miR-642a-5p/miR-532-3p/mir-329-5p, and circIPO11-

miR-659-3p/miR-424-5p/miR-106a-3p remains to be tested [71]. Another study in hepatitis B-related HCC revealed that circRNA-100338 acts as an endogenous sponge for miR-141-3p in regulating invasion potential of liver cancer [72]. A functional analysis in HCC revealed the role of circMTO1 in inhibiting HCC growth through sponging miR-9 resulted in the increased expression of p21 [109].

Role of circRNAs in molecular pathogenesis of basal cell carcinoma has also been demonstrated through the identification of 71 deregulated circRNAs and 354 potential miRNA response elements among these circRNAs in basal cell carcinoma [110]. Similarly, deregulation of circRNA expression has been demonstrated in malignancies such as oral, gastric, breast and cervical cancers [58].

4.5. CircRNA responses to radiation therapy and chemotherapy

Deregulation of circular RNA has also been linked to the acquired resistance to radiation therapy. For instance, 57 upregulated and 17 downregulated circRNAs (fold change ≥ 2.0 and $P < 0.05$) were found in radioresistant esophageal cancer cell line KYSE-150R compared to the parental KYSE-150 cell line [111]. An interaction analysis revealed correlations between circRNA-microRNA-mRNA as distinct interaction nodes [111]. Moreover, a comparison between seven acute myeloid leukemia (AML) patients undergoing standard induction chemotherapy at both without prior treatment and complete remission (CR) stage showed increased hsa_circ_0004277 [67]. This implies a potential restoration of dysregulated hsa_circ_0004277 expression after chemotherapy.

5. Role of circular RNA as biomarkers

The stability due to their cyclic structure and tissue specific expression of circRNAs make them a suitable candidate for biomarker studies [13,15]. CircRNAs are secreted and transported in human exosomes where they are abundant and stable [112]. In colorectal cells (CRC), expression of circRNAs has been detected in cells as well as in exosomes secreted by three CRC cell lines differing in KRAS mutation status [80]. Mammalian brain has been shown to have abundant circRNAs which are conserved in sequence and expression [113]. Analysis of RNA-seq data of human whole blood samples has revealed reproducible and abundant expression of circRNAs making them amenable for routine blood sample testing [114]. Similarly, RNA-seq with in depth bioinformatics analysis in human cell-free saliva samples validated the expression of circRNAs [115]. These biological properties make circRNAs promising biomarker candidates for diagnosis, prognosis and detection of cancer from blood and saliva samples.

In gastric cancer for example, the expression of hsa_circRNA_002059 has been correlated with distal metastasis, TNM stage, gender and age and therefore it has been proposed to be a biomarker for the diagnosis [81]. Similarly, in esophageal squamous cell carcinoma, hsa_circRNA_0067934 has been associated with poor differentiation and promote proliferation [85]. It can plausibly serve as a novel biomarker for disease progression or as a therapeutic target [85]. In bladder carcinoma, circTCF25 has been suggested to be a promising biomarker [68]. Research to validate the expression of these biomarkers in easily available specimens such as blood and saliva will increase the utility of these biomarkers.

6. Current limitations and challenges

Despite the major progress made this far, several limitations and challenges need to be addressed. A standard naming system is required to standardise circRNAs. Annotation of each circRNAs would be useful for further research on specific circRNA species [116]. In addition, one of the major limitations in circRNA studies is that most publicly available RNA-seq data sets related to cancer were prepared using a poly (A) purification step to enrich mRNA [116]. This might reduce the possibility of identifying circRNAs, which naturally lack poly (A) tails. Furthermore, genome wide studies were performed using microarrays or limited number of tissue samples, partly due to the lack of experienced pathologist in tumor tissue examination [116]. In most circRNAs discoveries involving cancer, unbiased RNA-seq is employed. However, it should be noted that the RNA quality and sample handling during RNA-seq library preparation step affects the discovery of circRNAs of different types and sizes. Variation in RNA-seq preparation will alter circRNAs detection, for example, size selection excludes small circRNA while oligo dT favors linear mRNA and biases against circRNAs [116,117]. Another challenge for the discovery of circRNA involves the detection and quantification of circRNA. The study of circRNAs may be hampered by template switching and rolling circle amplification during reverse transcription and by amplification bias during PCR [117,118].

7. Concluding remark

In conclusion, recent investigations in circRNAs and their roles in cancer development and progression are still limited. However, constant improvement of circRNA bioinformatics pipelines and application of new techniques will eventually pinpoint the roles of circRNAs beyond acting as miRNA sponges. Better understanding of the regulation of circRNAs' aberrant expression, circRNA/miRNA/mRNA networks and sponging activity would lead to robust biomarker development for cancer detection and clinical prognosis. Finally, further studies on this ancient RNA will provide a significant new perspective in cancer biology.

Abbreviations

ADM	adriamycin
AML	acute myeloid leukemia
ccRCC	clear cell renal cell carcinoma
circRNA	circular RNA
CR	complete remission
CRC	colorectal cancer
CRR	chemoradiation resistance
ESCC	esophageal squamous cell carcinoma
f-circRNA	fusion circRNA
GC	gastric cancer
HCC	hepatocellular carcinoma
KO	knockout
miRNA	micro RNA
PDAC	pancreatic ductal adenocarcinoma
RBP: RNA	binding protein

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Authors' contributions

Ng. W.L., Mohidin. T.B.M., and Shukla. K., perceived the ideas, collected the related papers and wrote the manuscript.

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