Am J Cancer Res 2018;8(7):1142-1156
www.ajcr.us /ISSN:2156-6976/ajcr0080004

Review Article

Circular RNAs in digestive system cancer: potential biomarkers and therapeutic targets

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Received May 21, 2018; Accepted June 22, 2018; Epub July 1, 2018; Published July 15, 2018

Abstract: Circular RNAs (circRNAs) are a series of special closed circular RNA molecules with stability and conservatism. In recent years, advances in high-throughput RNA sequencing technology have led to explosive discovery of circRNAs in different types of species and cells. Moreover, circRNAs can accomplish a remarkable multitude of biological functions, such as regulating transcription or splicing, serving as miRNA sponges, interacting with RNA-binding proteins, and translating proteins. Meanwhile, circRNAs involve in the biogenesis and development of many diseases, including cardiovascular disorders, nervous system disorders, cancers, etc. Herein, we discuss the latest research progress of circRNA, as well as their diagnostic and prognostic significance in digestive system cancers. In addition, this paper highlights that circRNAs might serve as potential therapeutic targets for novel drugs by taking digestive system cancer as an illustrative example.

Keywords: Circular RNA, digestive system cancer, diagnosis, prognosis, therapeutic targets

Introduction

Circular RNA (circRNA) is a special single-stranded closed circular RNA molecule in nature. It was first found in viroid as early as in 1976 [1], and was later isolated from several eukaryotes in 1979 [2]. Its biological significance kept unknown until scientists gradually unveiled its mystery with the help of high-throughput sequencing technology and bioinformatics in the 21st century. And the researches on circRNAs have seen explosive growth since 2012. Using innovative methods (for example, RNA-seq [3], RPAD [4], and circBase [5]), scientists have identified thousands of circRNAs in human beings [6], plants [7], and some animals [6, 8, 9]. Unlike linear RNAs, circRNAs, as a result of their special structure, are more stable, conserved, highly abundant, and dynamically expressed in a spatio-temporal manner inside particular tissues [6, 8-12]. Because of these biological properties, circRNAs may play significant roles as special biomarkers in occurrence and development of diseases. In recent years, circRNAs have gradually been demonstrated to correlate with various human diseases, including cardiovascular disorders [13-15], nervous system disorders [16, 17], intervertebral disc degeneration [18], silicosis [19], diabetic retinopathy [20], and cancers [21-26]. They also can serve as novel therapeutic targets for above diseases [14, 16, 18, 27]. This review comprehensively and concisely summarizes the biogenesis and function of circRNAs, and describes the application of main circRNA databases. Furthermore, we mainly discuss the emerging roles of circRNAs in digestive system cancers. Finally, the paper especially highlights that circRNAs might serve as potential therapeutic targets for novel drugs by taking digestive system cancers as an illustrative example.

The biogenesis of circRNAs

Unlike linear RNAs generated from canonical splicing, circRNAs usually arise from back-spliced events of precursor mRNAs (pre-mRNAs) and circularization of exons, introns, or both exons and introns [28]. Besides, canonical pre-mRNA splicing can compete against exon circularization in a tissue-specific and conserved manner from flies to humans [29-31]. Typically, a circular RNA splice junction is

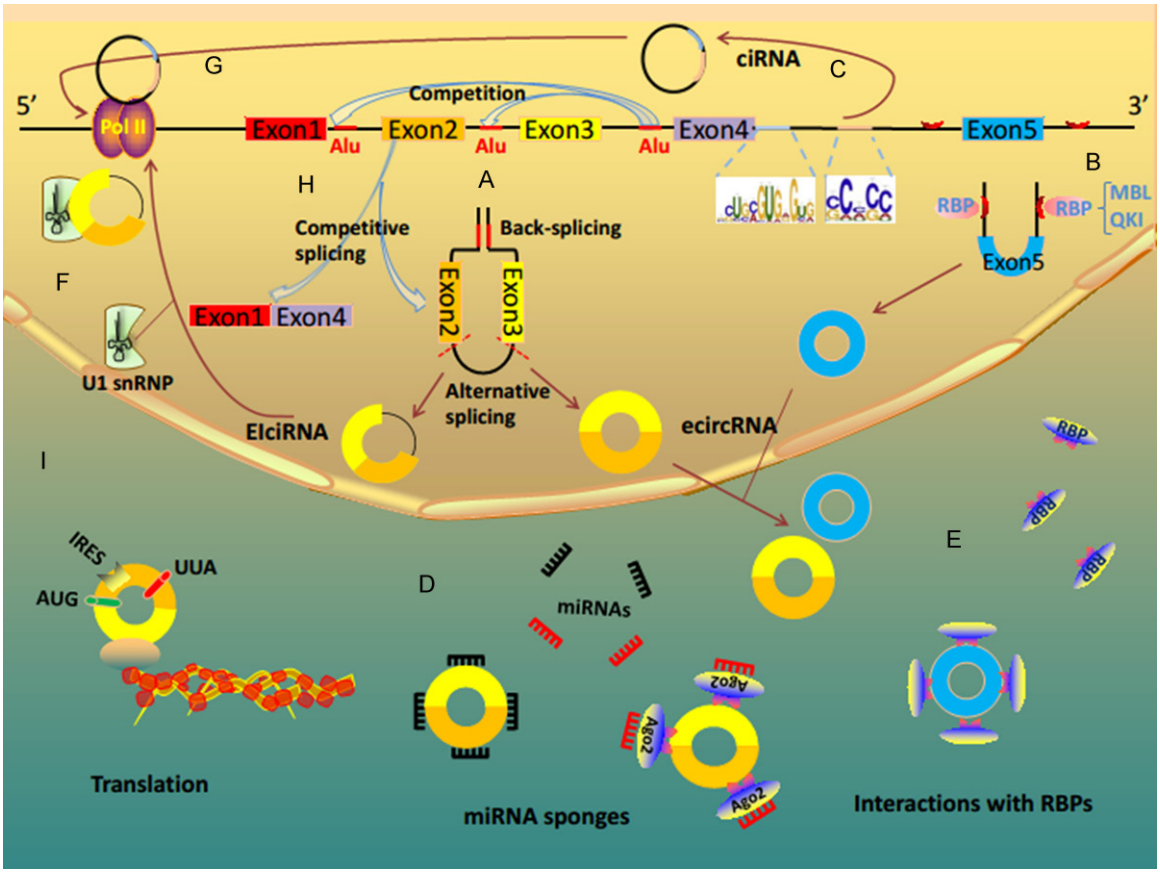

Figure 1. CircRNA biogenesis and function. A. The formation of reversely repeated Alu pairs and the competition between different Alu pairs leading to alternative circularization. During the procession of circularization, pre-mRNAs form ecircRNA or EIciRNA by removing or retaining intron respectively. B. EcircRNA biogenesis can be regulated by RNA-binding proteins, such as MBL and QKI. C. CiRNAs are derived from introns. They depend on a consensus motif containing a 7 nt GU-rich element near the 5' splice site and an 11 nt C-rich element close to the branchpoint site. D. CircRNAs serve as miRNA sponges to inhibit miRNA activity. E. Some circRNAs function as protein sponges and interact with RNA binding proteins (RBPs). F. Some EIciRNAs regulate Pol II transcription of their parental genes via specific RNA-RNA interaction between U1 snRNA and EIciRNAs. G. Some ciRNAs function as positive regulators of Pol II transcription and play a vital part in the efficient transcription of their parent coding genes. H. CircRNAs can modulate linear splicing by competing for splice sites of pre-mRNA. The formation of circRNA affects the alternative splicing of pre-mRNA, generating circRNA and corresponding linear RNA. I. Some circRNAs have protein-coding capacity and can translate proteins.

formed by a donor site at a downstream 3' end joining with an acceptor splice site at the 5' end of an exon (head-to-tail) [6, 32]. In human cells, ecircRNAs (exonic circRNAs) contain one or more exons. They account for the main part of circRNAs, and play an important role in many complicated yet critical biological functions [6, 33, 34]. One mechanism of ecircRNAs biogenesis is exon circularization mediated by flanking intronic complementary sequences. Noticeably, the formation of reversely repeated Alu pairs and the competition between different Alu pairs can lead to alternative circularization, producing multiple circular RNA transcripts from a single gene (**Figure 1A**) [35]. In another mechanism, ecircRNAs biogenesis can be regulated by RNA-binding proteins (RBPs) (**Figure 1B**) [29, 30]. Taking circMbl as an example, muscleblind (MBL), as a regulatory factor, can promote circMbl biogenesis. It depends on the presence of functional MBL binding sites in the flanking intronic sequences [29]. Quaking (QKI) is another RBP that strikingly facilitates circRNA formation via pre-mRNA binding sites. During human epithelial-mesenchymal transition (EMT), over one third of circRNAs abundance is dynamically regulated by QKI. It is dependent on intronic QKI binding motifs [30]. In addition, scientists

have also identified three other special subclasses of circRNAs with different biogenesis mode: circular intronic RNAs (ciRNAs) [36], exon-intron circRNAs (EIciRNAs) [37], and tRNA intronic circRNAs (tricRNAs) [31]. Unlike ecircRNAs, EIciRNAs retain the introns instead of removing them during splicing process. Moreover, some pre-mRNAs contain flanking Alu complementary pairs, while others contain flanking complementary sequence pairs other than Alu. Both of them facilitate the production of EIciRNAs (**Figure 1A**) [37]. Interestingly, ciRNAs derive from introns and accumulate due to a failure in debranching. Their formation depends on a consensus motif containing a 7 nt GU-rich element near the 5' splice site and an 11 nt C-rich element close to the branchpoint site (**Figure 1C**) [36]. TricRNAs are a special type of intronic circRNAs generating during pre-tRNA splicing [31]. Their expression is regulated in an age-dependent and tissue-specific manner. Biogenesis of tricRNAs requires anciently conserved tRNA sequence motifs and processing enzymes.

Function of circRNAs

As miRNA sponges: yes or no?

The hallmark function of circRNAs is their ability to serve as miRNA sponges (**Figure 1D**). The first observation of a circRNA acting as a miRNA sponge is ciRS-7 (also termed as CDR1). It has more than 70 conserved binding sites of miRNA-7. Thus, ciRS-7 likely plays profound roles in diseases such as cancer by fine-tuning the miR-7/miR-671/ciRS-7 axis [6, 38, 39]. Analogously, experiments with circular SRY RNA uncovered its function as a miR-138 sponge [39]. Since then, there have been more and more studies on the function of circRNAs as miRNA sponges. For example, circ-Foxo3, totally with 25 miRNA-binding sites, functions as a sponge for eight miRNAs. The ectopic expression of Foxo3 circRNA could suppress tumor growth, cancer cell proliferation and survival [40]. Similarly, circHIPK3 serves as a modulator of cell growth by sponging multiple miRNAs (9 miRNAs with 18 binding sites) in human cells [11]. These results indicate that one circRNA might be associated with a variety of miRNAs. But unlike ciRS-7, circHIPK3 only have 1-5 binding sites for one miRNA. Consistent with previous findings, circRNA HRCR was also proved to serve as a miR-223 sponge to regulate cardiac hypertrophy and heart failure [14]. Maybe it is a general phenomenon for circRNAs served as miRNA sponges.

However, as a special novel type of endogenous noncoding RNA, controversy on circRNAs is inevitable. For instance, Jeck et al. [41] showed that very few circRNAs in mammalian cells contained more than ten binding sites for an individual miRNA. Many exonic circRNAs just contained a smaller number of putative miRNA binding sites. Soon afterwards, Guo et al. [3] and Militello et al. [42] argued against the generalized properties that circRNAs act as miRNA sponges. Instead, they all held a view that most circRNAs do not function as miRNA sponges. Since most circRNAs are of low abundance and in small length, many circRNAs may not serve as miRNA sponges [11]. Therefore, it still remains to be illustrated whether miRNA sponges are widespread regulators of miRNA activity in eukaryotes, and how networks of circRNAs, miRNAs, and ceRNAs regulate cellular homeostasis.

Interaction with RBPs

In addition to the function of miRNA sponges, some circRNAs have a high density of binding sites for a single or multiple RBPs. They may function as protein sponges interacting with RBPs (**Figure 1E**) [43]. A typical example is ciR-7/CDR1as, widely associating with Argonaute (AGO) proteins in a miR-7-dependent manner [39]. Likewise, due to the presence of functional MBL binding sites in circMbl, MBL can directly bind to circMbl and promote circMbl production. Downregulation of MBL in both cultured cells and fly neural tissue leads to a remarkable decrease in circMbl production [29]. Another study revealed a competitive phenomenon between a circRNA and its homologous RBP mRNA. The extensive binding of circPABPN1 to HuR prevents HuR binding to PABPN1 mRNA and lowers PABPN1 translation [44]. Circ-Foxo3, encoded by the Foxo3 gene [45], appears to have tumor suppressive activity like its parental gene. Circ-Foxo3 is able to bind with 5 types of proteins including cyclin-dependent kinase 2 (CDK2) and cyclin-dependent kinase inhibitor 1 (p21) with a high affinity. Ectopic expression of circ-Foxo3 can suppress cell proliferation and block cell cycle progres-

sion, because it can bind to the cell cycle proteins CDK2 and p21 to form a circ-Foxo3-p21-CDK2 ternary complex [46].

Regulating transcription and splicing

EIciRNAs, predominantly localized in the nucleus, may have the function of gene transcription regulation [28, 36, 37]. For example, circEIF3J and circPAIP2 might regulate the Pol II transcription of their parental genes via specific RNA-RNA interaction between U1 snRNA and EIciRNAs (**Figure 1F**) [37]. CiRNAs, another subclass of circRNAs, can also regulate gene expression. They are abundant in the nucleus but have few miRNA target sites. Some ciRNAs, such as ci-ankrd52 and ci-sirt7, function as positive regulators of Pol II transcription and play an important role in the efficient transcription of their parent coding genes (**Figure 1G**) [36].

CircRNAs also modulate linear splicing by competing with splice sites of pre-mRNA. Thus, the formation of circRNA affects the alternative splicing of such pre-mRNA, potentially generating circRNA and corresponding linear RNA (**Figure 1H**) [29, 34]. As previously described, MBL can bind directly to circMbl and promote circMbl production. Thus, it negatively affects canonical splicing and decreases the production of the parental mRNA [29]. Additionally, abundant ecircRNAs derive from exon 2 of HIPK2/3 undergoing "alternative" splicing to circularize rather than canonical splicing to produce linear transcript. Circularization of HIPK2/3 negatively regulates the usual protein-encoding transcript production [12].

Translating proteins

Translating proteins is another amazing discovery about the biological function of circRNAs (**Figure 1I**), which is contrary to the conventional concept of circRNAs [32, 47]. Previously, protein-coding process was thought to merely transfer from DNA to a linear mRNA then to a protein. CircRNA was regarded as a distinct class of endogenous noncoding RNA, because it is lack of a 5' 7-methylguanosine cap structure as well as a poly (A) tail. Both of them are required for efficient translation of linear mRNAs [48]. But as early as 1995, eukaryotic ribosomes was reported to initiate translation on circRNAs in vitro, as RNAs contain internal ribosome entry site (IRES) elements [49]. Besides, covalently closed circular (CCC) RNA is the smallest (220 nt) RNA among all known viroids and virusoids. In 2014, the CCC RNA was found to contain an IRES element and was directly translated by eukaryotic ribosomes through two (or three) completely overlapping open reading frames (ORFs) [50]. Since then, more and more convincing studies have emerged that circRNAs may be used as templates for protein synthesis [51-57].

So far, at least four studies have offered sufficient proofs for the existence of translatable circRNA molecules: circMbl [56], circ-ZNF609 [57], circ-FBXW7 [53], and circ-SHPRH [54]. Circ-ZNF609 contains an ORF spanning from the start codon and terminating at an in-frame STOP codon. It can be translated into a protein in a splicing-dependent and cap-independent manner [57]. Analogously, circMbl can also be translated in a cap-independent way in drosophila heads. Furthermore, starvation and FOXO likely regulate the translation of a circMbl isoform [56]. Additionally, circ-SHPRH [54] and circ-FBXW7 [53], as well as proteins coded by them, are found to be abundantly expressed in normal human brains while down-regulated in glioma. Both of the circRNAs have an ORF driven by the IRES to translate a functional protein. The translation from circRNA may be prevalent in other cancers, as a question to be addressed in the future.

Different from these individual examples, N6-methyladenosine (m6A), the most abundant base modification of RNA, was reported to promote efficient initiation of protein translation from circRNAs [55]. The phenomenon is widespread in human cells. Hundreds of endogenous circRNAs have translation potential. Meanwhile, circRNA translation initiated by m6A is heightened under heat shock condition. It is indicated that circRNA-encoded proteins may play important roles in cellular responses to environmental stress.

All of the above researches suggested that endogenous circRNAs may generate proteins. It expands the coding landscape of human transcriptome, and provides a new direction for circRNA research in the future.

CircRNA databases

With the application of next-generation sequencing and bioinformatics, numerous cir-

Table 1. Databases of circRNAs

Database	URL	Description	Ref.
CircBase	http://www.circbase.org/	Provides merged and unified datasets of circRNAs, with evidence supporting their expression	[5]
CircNet	http://circnet.mbc.nctu.edu.tw/	Provides information on known and novel circRNAs, circRNA-miRNA-gene regulatory networks	[66]
CircInteractome	https://circinteractome.nia.nih.gov/	Provides bioinformatic analyses of binding sites on circRNAs, identifies potential circRNAs acting as RBP sponges and potential internal ribosomal entry sites, designs primers or siRNAs for specific circRNAs	[65]
CircRNADb	http://reprod.njmu.edu.cn/circrnadb/	Annotates protein-coding potential of each circRNA	[64]
Circ2Traits	http://gyanxet-beta.com/circdb/	Predicts interactions among the miRNAs, protein coding, long non-coding and circular RNA genes, checks the enrichment of genes associated with particular biological processes by analyzing protein coding genes in the miRNA-circRNA interactome of individual diseases, and identifies disease associated SNPs on circRNA loci and Argonaute (Ago) interaction sites on circular RNAs	[60]
starBase v2.0	http://starbase.sysu.edu.cn/	Predicts interaction networks between lncRNAs, miRNAs, circRNAs, mRNAs, and RBPs from large-scale CLIP-seq data	[59]
deepBase v2.0	http://deepbase.sysu.edu.cn/	Provides comprehensive expression and evolution profiles of lncRNAs, circRNAs and small RNAs, identifies and annotates lncRNAs and circRNAs	[58]
CIRCpedia	http://www.picb.ac.cn/rnomics/circpedia/	Identifies and annotates back-splicing and alternative splicing of circRNAs in different cells	[111]
TSCD	http://gb.whu.edu.cn/TSCD/	Provides a new integrated view to explore the tissue-specific circRNAs in human and mouse tissues	[61]
CSCD	http://gb.whu.edu.cn/CSCD	Provides an integrated circRNA database to promote functional studies of cancer-specific circRNAs	[62]
CircPro	http://bis.zju.edu.cn/CircPro	An integrated tool capable of detecting circRNAs with protein-coding potential from high-throughput sequencing data	[63]

cRNAs are being discovered. They are integrated into the circRNA database for more thorough researches (**Table 1**). These databases contain a mass of circRNA genomes and mature RNA sequences of different species, as well as circRNAs relating to various diseases [5, 58-62]. Hence, they provide a transparent and comprehensive view of the spatio-temporal presence and function of circRNAs. Some databases are capable of detecting circRNAs with protein-coding potential [63, 64], while others are able to predict interaction networks between noncoding RNAs, circRNAs, and RBPs [58-60, 65, 66].

Recent research progress of circRNAs in digestive system cancer

CircRNAs widely exist in normal human tissues and cells. They possess a variety of biological functions to regulate gene expression in a spatio-temporal and tissue-specific manner. The translation of circRNAs can be regulated under stress conditions [55, 56]. Thus, circRNAs might serve as potentially novel and promising biomarkers for the diagnosis, therapy, and prognosis of human diseases, especially cancer. This review mainly focuses on the recent research progress in digestive system cancer. The detailed information is summarized in **Table 2**.

Esophageal cancer

By analyzing a total of 684 esophageal squamous cell carcinoma (ESCC) and paired adjacent non-tumor tissue samples, researchers found that cir-ITCH may have an inhibitory effect on ESCC by regulating the Wnt pathway [67]. On the contrary, another study indicated that hsa_circ_0067934 was able to promote the proliferation and migration of ESCC cell lines [68]. Its expression was associated with tumor differentiation and tumor-node-metastasis (TNM) stage. Besides, the abnormal expression of circRNAs may play a role in radiotherapy resistance of esophageal cancer [69]. Recently, Sun et al. [70] provided the first global circRNA expression profiling of all the types of esophageal tumor cells by a combination of RNA sequencing and bioinformatics analysis. They found that circRNAs were involved in tumor metabolism, cell apoptosis, proliferation, and migration.

Gastric cancer

Gastric cancer is one of the commonest malignancies worldwide [71]. It lacks reliable and efficient early diagnostic biomarkers, therefore the 5-year overall survival (OS) rate remains low in advanced patients [72]. To improve the clini-

Table 2. CircRNAs in digestive system cancer: signaling pathway and function

circRNA	Cancer type	Expression in tumors	Pathway/regulatory network	Function/clinical association	Ref.
cir-ITCH	ESCC	Low	cir-ITCH-miR-7/miR-17/miR-214-ITCH-Wnt signaling	Reduces cell viability and arrests proliferation in ESCC cells	[67]
	Colorectal cancer	Low	cir-ITCH-Wnt signaling	Plays an anti-tumor role	[78]
	HCC	Low	Not mentioned	Has an inhibitory effect on HCC, and associates with survival of HCC	[112]
hsa_circ_006793	ESCC	High	Not mentioned	Correlates with tumor differentiation and TNM stage; promotes proliferation and migration of ESCC cell	[68]
circPVT1	Gastric cancer	High	circPVT1-miR-125-E2F2	An independent prognostic factors for overall survival and disease free survival in patients with gastric cancer; affects proliferation of gastric cancer cells	[24]
ciRS-7 (CDR1as)	Gastric cancer	High	ciRS-7-miR-7-PTEN/PI3K/AKT pathway	An independent risk factor of overall survival; Correlates with a more aggressive oncogenic phenotype	[73]
	Colorectal cancer	High	ciRS-7-miR-7-EGFR/RAF1/MAPK pathway	An independent risk factor for overall survival with tumor stimulative effect	[79]
	HCC	high	ciRS-7-miR-7-EGFR or PI3K/AKT/mTOR pathway	An independent risk factor of hepatic microvascular invasion; correlates with the proliferation of hepatocellular carcinoma cells and the deterioration of HCC	[83, 84]
hsa_circ_0001895	Gastric cancer	Low	Not mentioned	Associates with cell differentiation, Borrmann type, and tissue CEA expression; has a better sensitivity and specificity than the commonly used biomarkers of gastric cancer	[74]
circLARP4	Gastric cancer	Low	circLARP4-miR-424-LATS1-YAP pathway	An independent prognostic marker for overall survival of gastric cancer patients as well as the patients with chemotherapy; inhibits DNA synthesis, cell proliferation and invasion; correlates with tumor size and lymphatic metastasis	[75]
hsa_circ_0000745	Gastric cancer	Low	hsa_circ_0000745-miRNAs	Associates with tumor differentiation; has higher sensitivity and specificity than serum CEA levels	[76]
hsa_circ_000984	Colorectal cancer	High	hsa_circ_000984-miR-106b-CDK6	Promotes colorectal cancer cell proliferation, migration, invasion and tumor formation; correlates with advanced colorectal cancer	[81]
hsa_circ_001569	Colorectal cancer	High	hsa_circ_001569-miR-145-E2F5/BAG4/FMNL2	Promotes colorectal cancer cell proliferation and invasion	[82]
circCCDC66	Colorectal cancer	High	circCCDC66-miRNAs-oncogenes	Promotes colorectal cancer cell proliferation, migration, invasion, and anchorage-independent growth; associates with poor prognosis	[22]
circHIPK3	Colorectal cancer	High	c-Myb-circHIPK3-miR-7-FAK, IGF1R, EGFR, YY1	Positively correlated with metastasis and advanced clinical stage; an independent prognostic factor of poor overall survival in colorectal cancer	[80]
circMTO1	HCC	Low	circMTO1-miR-9-p21	Suppresses hepatocellular carcinoma cell proliferation and invasion; associates with survival of HCC	[26]
hsa_circ_0004018	HCC	Low	hsa_circ_0004018-miR-30e-5p/miR-626-MYC	Correlates with serum AFP level, tumor diameters, differentiation, BCLC stage and TNM stage; HCC-stage-specific expression features in diverse chronic liver diseases	[85]
circZKSCAN1	HCC	Low	circZKSCAN1-miRNA-Mrna	Associates with tumor numbers, cirrhosis, vascular invasion, or microscopic vascular invasion; inhibits growth, migration, and invasion of HCC	[86]
cSMARCA5	HCC	Low	cSMARCA5-miR-17-3p/miR-181b-5p-TIMP3	An independent risk factor for overall survival and relapse-free survival after hepatectomy; inhibits the proliferation and migration of hepatocellular carcinoma cells	[87]
circRNA_100338	HCC	High	circRNA_100338-miR-141-3p	Correlates with a low cumulative survival rate and metastatic progression in HCC patients with hepatitis B	[88]
circARSP91	HCC	Low	AR-ADAR1-CircARSP91	Suppresses tumor growth	[89]

cal outcome of patients, it's essential to identify effective biomarkers for early diagnosis. Several studies revealed that circRNA may act as a prognostic marker in gastric cancer.

CircPVT1, for example, is a novel proliferative factor and prognostic marker in gastric cancer. It was upregulated in patients with gastric cancer [24]. But surprisingly, high levels of circPVT1 predicated a significantly better overall survival in gastric cancer patients than those with low levels of circPVT1. It may be explained by the positive association of circPVT1 with the tumor suppressor miR-125. Analogously, ciRS-7 was significantly upregulated in gastric cancer tissues compared with the normal tissues. The upregulation of ciRS-7 was linked to poor survival and was probably an independent risk factor of overall survival [73]. Overexpression of ciRS-7 in MGC-803 and HGC-27 cells led to a more aggressive oncogenic phenotype through resisting miR-7-mediated PTEN/PI3K/Akt pathway with tumor-suppression effect. Recently, three independent studies respectively found that hsa_circ_0001895 [74], circLARP4 [75], and hsa_circ_0000745 [76] were relatively downregulated in gastric cancer tissues compared to adjacent noncancerous tissues. Hsa_circ_0001895 was decreased not only in gastric cancer tissues but also in gastric precancerous lesions [74]. It was significantly correlated with cell differentiation, Borrmann type, and tissue carcinoembryogenic antigen (CEA) expression. CircLARP4 inhibited biological behaviors of gastric cancer cells by sponging miR-424. It represented an independent prognostic factor for overall survival of gastric cancer patients [75]. Hsa_circ_0000745 was also down-regulated in plasma samples from patients with gastric cancer. The expression level in gastric cancer tissues and plasma was correlated with tumor differentiation and TNM stage respectively. Additionally, combined with CEA level, plasma hsa_circ_0000745 level is a more promising diagnostic marker for this malignancy [76]. Interestingly, a four-circRNA-based classifier model, established by Zhang et al., could predict the early recurrence of stage III gastric cancer after radical surgery. Its accuracy can be further improved by combining with traditional TNM staging [77]. In conclusion, circRNAs play crucial roles during gastric cancerogenesis and are potential biomarkers for clinical prognosis prediction. What's more, tissue-specific expression of circular RNAs combined with the commonly used biomarkers may contribute to improving the sensitivity and specificity in the screening of gastric cancer.

Colorectal cancer

During the development of colorectal cancer, some circRNAs serve as oncogenes, while others function as anti-oncogenes. For instance, cir-ITCH was down-regulated in colorectal cancer compared with the peritumoral tissue and was related with Wnt/β-catenin pathway [78]. However, ciRS-7 was obviously upregulated in colorectal cancer. Its overexpression inhibited miR-7 and subsequently activated EGFR and RAF1 oncogenes. CiRS-7 emerged as an independent risk factor for overall survival [79]. More recently, circHIPK3 was found to be significantly upregulated in colorectal cancer tissues and cell lines [80]. It positively correlated with metastasis and advanced clinical stage. Moreover, high-level expression of circHIPK3 was an independent prognostic factor of poor overall survival in colorectal cancer. There are also several other circRNAs serving as oncogenes in colorectal cancer, such as hsa_circ_000984 [81], hsa_circ_001569 [82], and circCCDC66 [22]. On one hand, all of them promoted colorectal cancer cells proliferation, invasion [82], and even metastasis [22, 81]. On the other hand, they exerted functions via circRNA/miRNA/targets axis [81, 82] or directly regulating a subset of oncogenes [22].

Hepatocellular carcinoma (HCC)

HCC is the commonest malignancy of liver leading to death due to high frequency of metastasis and recurrence [71]. It is urgent to identify potential biomarkers and to find new targets for designing more effective therapies. Recent years, numerous miRNAs and long noncoding RNAs (lncRNAs) have been found to closely relate with the occurrence of HCC. Though the regulation and function of circRNAs in HCC remains largely unknown, there is mounting evidence that circRNAs are involved in HCC development.

CiRS-7 (also termed as CDR1as) is the best-known circRNA so far functioning as a sponge for miR-7 [6]. It has been mentioned in gastric cancer and colorectal cancer above. Researchers found that the overexpression of ciRS-7 in HCC was significantly correlated with hepatic microvascular invasion (MVI), alpha fetal protein (AFP) level, and younger age, thus it might be related with the deterioration of HCC [83].

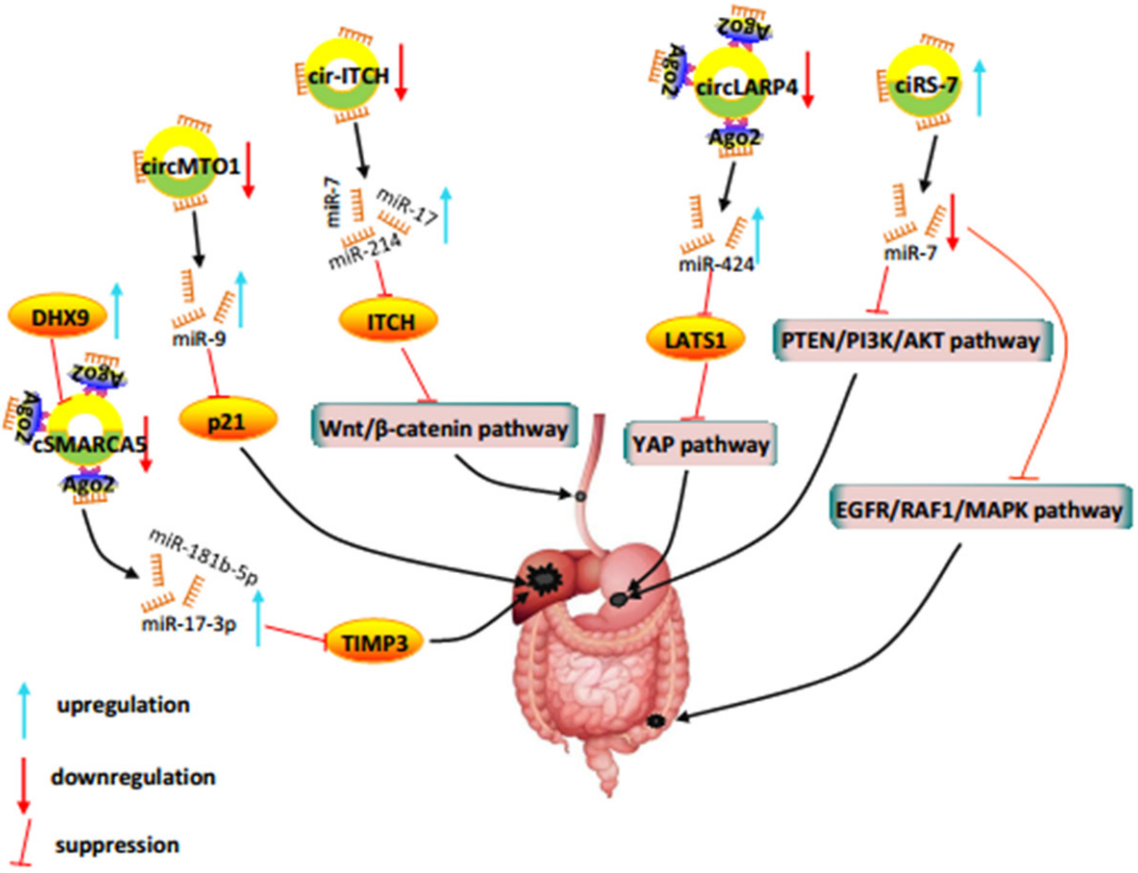

Figure 2. CircRNA-mediated mechanisms in digestive system cancer. CircRNA functions as the sponge of miRNAs. The expression dysregulation of circRNA will affect the expression of related miRNA target genes via upregulation or downregulation of free miRNA, ultimately giving rise to cancer.

Subsequently, ciRS-7 was revealed to exert its effects on cell proliferation by regulating epidermal growth factor receptor (EGFR) expression via targeting miR-7 in HCC cells [84]. Additionally, several circRNAs with differential expression have been identified in liver cancer. Taking circMTO1 as an example, it was significantly down-regulated in HCC [26]. CircMTO1 held back HCC progression through acting as the sponge of oncogenic miR-9 to promote p21 expression. Low-level expression of circMTO1 in HCC tissues may be a prognosis predictor for shortened survival of patients. Similarly, hsa_circ_0004018 also decreased obviously in HCC tissues [85]. It was correlated with serum AFP level, tumor diameters, differentiation, Barcelona Clinic Liver Cancer (BCLC) stage, and TNM stage. More importantly, hsa_circ_0004018 showed stage-specific expression features in diverse chronic liver diseases from chronic hepatitis, cirrhosis to HCC. It may play an important part in the carcinogenesis and metastasis of HCC. Interestingly, another study manifested that ZKSCAN1 gene and its related circRNA (circZKSCAN1) inhibited HCC cell growth, migration, and invasion through different downstream signaling pathways [86]. Recently, circRNA cSMARCA5 was found to promote the expression of TIMP3, a well-known tumour suppressor, via sponging miR-17-3p and miR-181b-5p [87]. It could inhibit the proliferation and migration of HCC. Furthermore, the down-regulation of cSMARCA5 in HCC was strongly associated with invasion. It might serve as an independent risk factor for overall survival and recurrence-free survival (RFS) after hepatectomy in HCC patients. In addition, circRNA_100338, highly expressed in HCC, was closely correlated with a low cumulative survival rate and a high metastasis rate in HCC patients with hepatitis B [88]. Finally, androgen receptor (AR), a transcriptional activator of

ADAR1 promoter, was found to suppress circRNA expression by upregulating ADAR1. More significantly, ADAR1 expression could effectively predict prognosis of HCC patients and was positively correlated with AR in HCC. At the same time, circARSP91, downregulated by AR in an ADAR1-dependent manner, could inhibit HCC tumor growth [89]. The results provided an explanation about the obvious gender bias of HCC from a new angle. The rapidly growing interest and emerging data are likely to provide new insights for the research of circRNA and help to identify novel candidates for biomarkers or therapeutic intervention.

Therapeutic approaches targeting circRNAs in digestive system cancer

Differential expression of circRNA has been reported in tumors compared with normal tissues. The identification of circRNA contributing to tumor suppression or tumorigenesis provides opportunities to develop novel therapeutics for cancer [11, 46, 53]. The relationship between various novel circRNAs and carcinogenesis signaling pathways indicates that circRNAs are potential therapeutic targets for novel drugs in digestive systerm cancer (**Figure 2**). For example, overexpression of ciRS-7 could inhibit miR-7-mediated tumor suppression via antagonizing miR-7-mediated PTEN/PI3K/Akt pathway in gastric cancer [73] and EGFR/RAF1/MAPK pathway in colorectal cancer [79]. Furthermore, the expression of circRNA is regulated by Pol II promoters, hence disease-activated control elements can be used to limit circRNA expression in malignant cells, while cell-specific promoters can be used to control circRNA expression in certain cell types [90]. Gene knockout is another classical approach to cause circRNA function deficiency, thus using this technology may remove the carcinogenic circRNA. Rajewsky et al. have successfully created an *in vivo* loss-of-function model from the mouse genome by CRISPR/Cas9 system targeting Cdr1as [33]. It offers an unprecedented opportunity for removing oncogenic circRNA. Another example is circLARP4. It can upregulate the expression of large tumor suppressor kinase 1 (LATS1) by sponging miR-424 and AGO2 protein. Then the LATS1 downstream effectors were downregulated to suppress gastric tumorigenesis and progression [75]. This unique cellular capacity of circRNA to sponge miRNA and proteins also makes it a promising therapeutic vector. Exogenous introduction of synthetic circRNA with multiple binding sites for specific oncogenic miRNA and/or proteins, namely artificial sponges, may restore the normal regulatory network to control proliferation or induce apoptosis in cancer cells [34]. Moreover, circRNA sponge will show more potent anti-cancer effect than linear miRNA sponge [91] due to its potentially low off-target effect as well as its specific and stable structure. Ultimately, since circRNAs can modulate the stability of some proteins like p53 [92] and STAT3 [93] through circRNA-miRNA-mRNA axis to participate in antitumor immunity, circRNAs may be a promising target for tumor immunotherapy [94]. In addition, because of the stable and specific properties of circRNAs, they may serve as tumor antigens for immune response [94-96]. For example, purified circRNAs can activate innate immunity and be recognized by RIG-I. Therefore, foreign circRNAs from tumor cells may also activate immunocytes to fight tumors.

Future perspectives

CircRNAs were previously thought to be junk byproducts in the process of gene transcription [97, 98]. However, accumulating researches in different cell types and tissues were performed to explore underlying functions of circRNAs. CircRNAs were confirmed to accomplish a remarkable multitude of biological functions such as regulating transcription or splicing, serving as miRNA sponges, interacting with RBPs, translating proteins, and so on. They are also involved in the biogenesis and development of many diseases, especially in cancer. Furthermore, some tools have been developed to annotate circRNAs [99, 100], such as circRNA_finder [101], CIRCexplorer [35], find_circ [6], UROBORUS [102], KNIFE [103], CIRI [104], MapSplice [105], segemehl [106], and NCLscan [107]. These tools help to expand our understanding of the complex information regarding circRNAs in eukaryotic transcriptomes. As well, these tools were suggested to be used combinedly to improve the sensitivity and specificity of circRNA identification. Despite such useful state-of-the-art circRNA detection tools exist, there is still a lack of a standardized naming system for circRNA research.

More remarkably, chromosomal translocation sometimes generates fusion genes with onco-

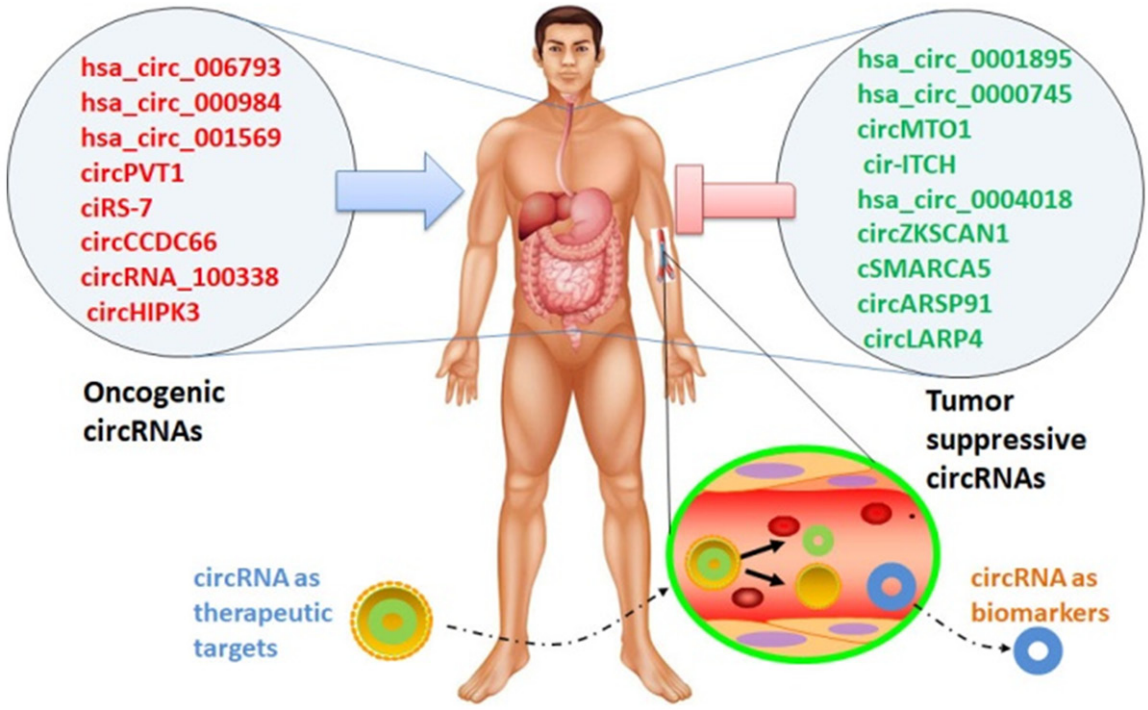

Figure 3. Cancer-promoting and cancer-suppressing circRNAs in digestive system cancers. Emerging evidences have demonstrated that circRNA expression is dysregulated in digestive system cancers. Different circRNAs play different roles, and they are antagonistic to each other. Some circRNAs may play oncogenic roles, whereas others play tumor suppressive roles in cancer. Moreover, circRNAs may serve as potential therapeutic targets for novel drugs and potential biomarkers for diagnosis and prognosis.

genic potential [108]. The phenomenon has been described in many hematological and solid malignancies. In addition to creating coding fusion mRNAs, cancer-associated chromosomal translocation could result in aberrant formation of circular RNAs, namely fusion circRNAs (f-circRNA) [109, 110]. Moreover, f-circRNAs, with tumor-promoting properties, promote cell viability and resistance upon therapy, and contribute to cellular transformation. Nevertheless, there is still limited knowledge about the underlying mechanisms of f-circRNA to drive tumorigenesis and it still needs to be elucidated in more details.

Taken together, these studies expand the current knowledge and add complexity regarding molecular mechanisms involved in cancer occurrence and progression. New candidate cancer-associated circRNA genes are being identified and their molecular mechanisms are being elucidated. CircRNAs offer new possibilities for understanding cancer pathogenesis. As previously reviewed, many circRNAs have been verified to be potential markers of diagnosis, progression, or prognosis in digestive system cancer (**Figure 3**). Besides, large numbers of novel circRNAs are likely to be identified in the near future. An advanced understanding for circRNA in digestive system cancer will undoubtedly provide beneficial insights and generate new hypothesis regarding disease pathogenesis, which might eventually take breakthroughs in clinical application.

Acknowledgements

This work was supported by State Key Laboratory of Cancer Biology (Fourth Military Medical University), No. CBSKL201720; Natural Science Foundation of Hubei Province, No. 2017CFB457; National Natural Science Foundation of China, No. 81372663 and No. 81772969.

Disclosure of conflict of interest

None.

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