

Review

Circular RNAs: Regulators of Cancer-Related Signaling Pathways and Potential Diagnostic Biomarkers for Human Cancers

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Abstract

Circular RNAs (circRNAs) are newly discovered endogenous non-coding RNAs featuring structural stability, high abundance, and tissue-specific expression. CircRNAs are prevalent and conserved in mammalian cells. They are involved in cellular processes and regulate gene expression at the transcriptional or post-transcriptional level by interacting with microRNAs (miRNAs) and other molecules. Recent studies have shown that circRNAs play an important role in the progression of various human diseases including atherosclerosis, nervous system disorders, diabetes, and cancer. In this review, we summarize the advances on endogenous circRNAs in eukaryotic cells and elucidate their diagnostic and prognostic significance in human cancers. Especially, we highlight the involvement of circRNAs in signal transduction pathways as well as their clinical potential to serve as biomarkers.

Key words: circular RNA; cancer; diagnosis; biomarkers.

Introduction

Circular RNAs (circRNAs) are newly discovered endogenous non-coding RNAs hundreds or even thousands of bases in length with a covalently closed structure [1, 2] and are transcribed by RNA polymerase II with the same efficiency as linear RNAs [3]. However, unlike in linear RNA splicing processes, circRNAs are produced when the splice donor site is joined to a splice acceptor site upstream in the primary transcript [4, 5]. Previous studies have demonstrated that circRNAs are tissue and cell-type specific, developmentally regulated, and exist during the eukaryotic tree of life suggesting they have important functions in various biological activities [6, 7]. Some circRNAs can be produced by the same gene with different isoforms showing different expression profiles, such as circSTAU2a and circSTAU2b [8].

CircRNAs are produced by circularization of a

single exon [9, 10], several exons [11], exons and introns named exon-intron circRNA (ElcircRNA) [12], or intronic sequences alone (circularized intron RNA; ciRNA) [13, 14]. CircRNAs were first found in some plant viroids as covalently closed circular RNA molecules with the base pairing between inverted complementary sequences at the 3' and 5' ends of linear molecules [15]. This was confirmed by an electron microscopic study which also indicated that circRNAs are mainly located in the cytoplasm of eukaryotic cells [16]. In 1986, Kos *et al.* discovered a single-stranded circular RNA molecule in the Hepatitis delta virus (HDV) by electron microscopy which was the first animal virus identified with a circular RNA genome [17].

A decade ago, Cocquerelle *et al.* identified novel human ets-1 transcripts which corresponded to

circular RNA molecules containing only exons in genomic order. These novel transcripts were structurally stable and localized in the cytoplasmic compartment of the cells. This new type of circRNAs was thought to represent a novel aspect of gene expression and hold some interesting clues about the splicing mechanism [18]. However, these new discoveries did not receive much attention and circRNAs were merely considered to be a class of low-abundance RNA molecules formed by mis-splicing of exon transcripts.

With the progress of deep sequencing of long RNAs and computational algorithms, a large number of novel circRNAs were identified in plants, invertebrates, and vertebrates regardless of whether they were replicated and/or induced *in vivo* accumulation of small RNAs [19, 20]. High-throughput sequencing analyses have shown that circRNAs are abundant and stable in eukaryotic transcriptomes suggesting that they may be the key players in the RNA world [7, 21-23]. CircRNAs exhibit tissue- and developmental-specific expression and play crucial roles in multiple cellular processes, such

as functioning as miRNA sponges by sequestering miRNA thereby affecting the stability of target mRNAs and dynamically regulating mRNA translation (Figure 1) [24-26]. Recently, Memczak *et al.* detected 1950, 1903, and 724 circRNAs in human, mice, and *C. elegans*, respectively [25]. Zheng *et al.* generated ribosomal-depleted RNA sequencing data from six normal tissues and seven cancers and detected at least 27,000 circRNA candidates [27]. They claimed that many of these circRNAs were differentially expressed between the normal and cancerous tissues. Recently, a new role for circRNAs as regulators of gene expression in the nucleus has been described. CircRNAs were reported to enhance the expression of their parental genes in cis and regulate the transcription activity of target genes via specific RNA-RNA interactions between U1 snRNA and ElcircRNAs [12]. Thousands of well-expressed, stable circRNAs were identified in various human diseases and life processes, such as aging [28-32], insulin secretion [33, 34], tissue development [35, 36], atherosclerotic vascular disease risk [37-39], cardiac hypertrophy [40-44], and cancer [26, 45-49].

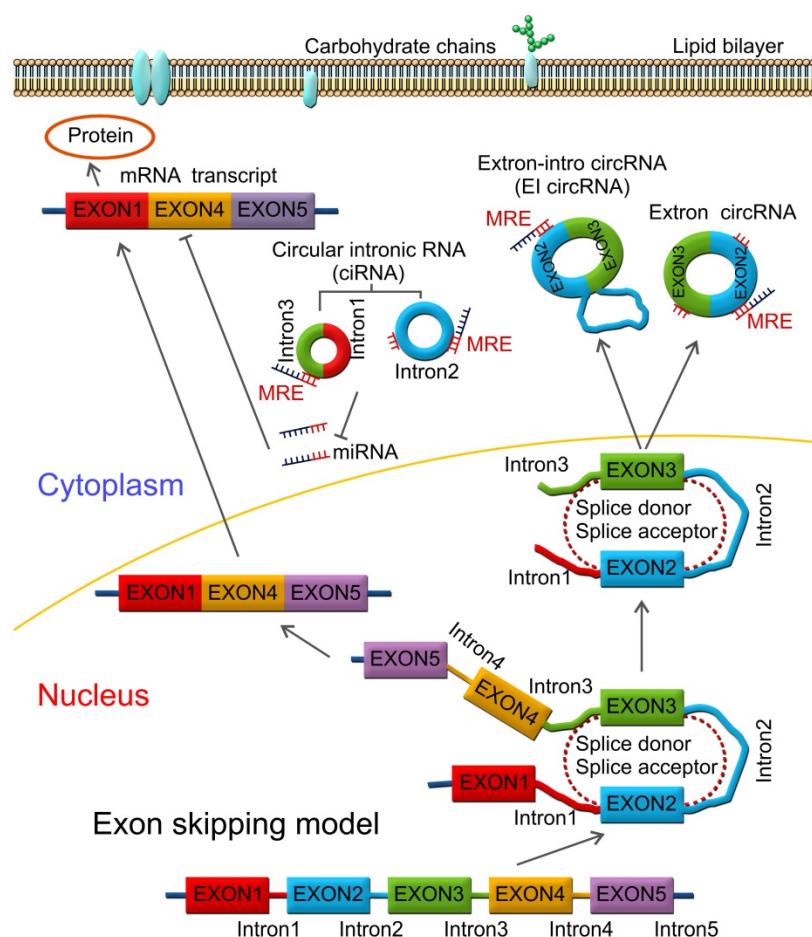


Figure 1. Lariat-driven circularization model. CircRNAs are produced by a lariat-driven circularization model containing exon 2 and exon 3 produced from exon skipping. The lariat subsequently undergoes internal splicing and circRNAs are generated with or without removing introns 1 and 3. The important roles of circRNAs include functioning as miRNA sponges and sequestering miRNAs by interacting with miRNAs via miRNA response elements (MRE), regulating mRNA translation and targeting mRNA stability.

Formation of circRNAs and key features

Pre-mRNAs are spliced into linear molecules that alternately join the exons and retain the exon order [50, 51]. The alternative splicing generates mRNAs encoding proteins [52-54]. Recently, it was shown that exon transcripts in pre-mRNAs might also be non-linearly reverse-spliced into a circular RNA [55-59]. There are two kinds of circRNAs: exonic circRNAs [60-62] and intronic circRNAs [63-65]. The biogenesis and formation of exonic circRNA mainly include three types of circularization driven by lariats [66, 67], intron-pairing [68, 69], and RNA-binding proteins (RBPs) [70, 71], which are described below.

Mostly, circRNAs are formed through lariat-driven circularization model (Figure 1). The

mechanism involves the creation of a lariat containing an exon produced from exon skipping. For example, the splice donor of 3'-end of exon 1 and splice acceptor of 5'-end of exon 4 are covalently joined together. The lariat is subsequently processed by internal splicing. Finally, the intron is released and the circRNA consisting of exon2-exon3 is formed.

In some other cases, circRNAs are formed via direct back splicing (Figure 2). Two unspliced introns interact by complementary base pairing, thereby juxtaposing the branch point of the 5'-intron and the 3'-intron-exon junction (3-splice donor) for a nucleophilic attack and cleavage. Then, the 3'-splice donor attacks the 5'-intron-exon junction (5-splice acceptor) joining the two introns and releasing the circularized exon [72].

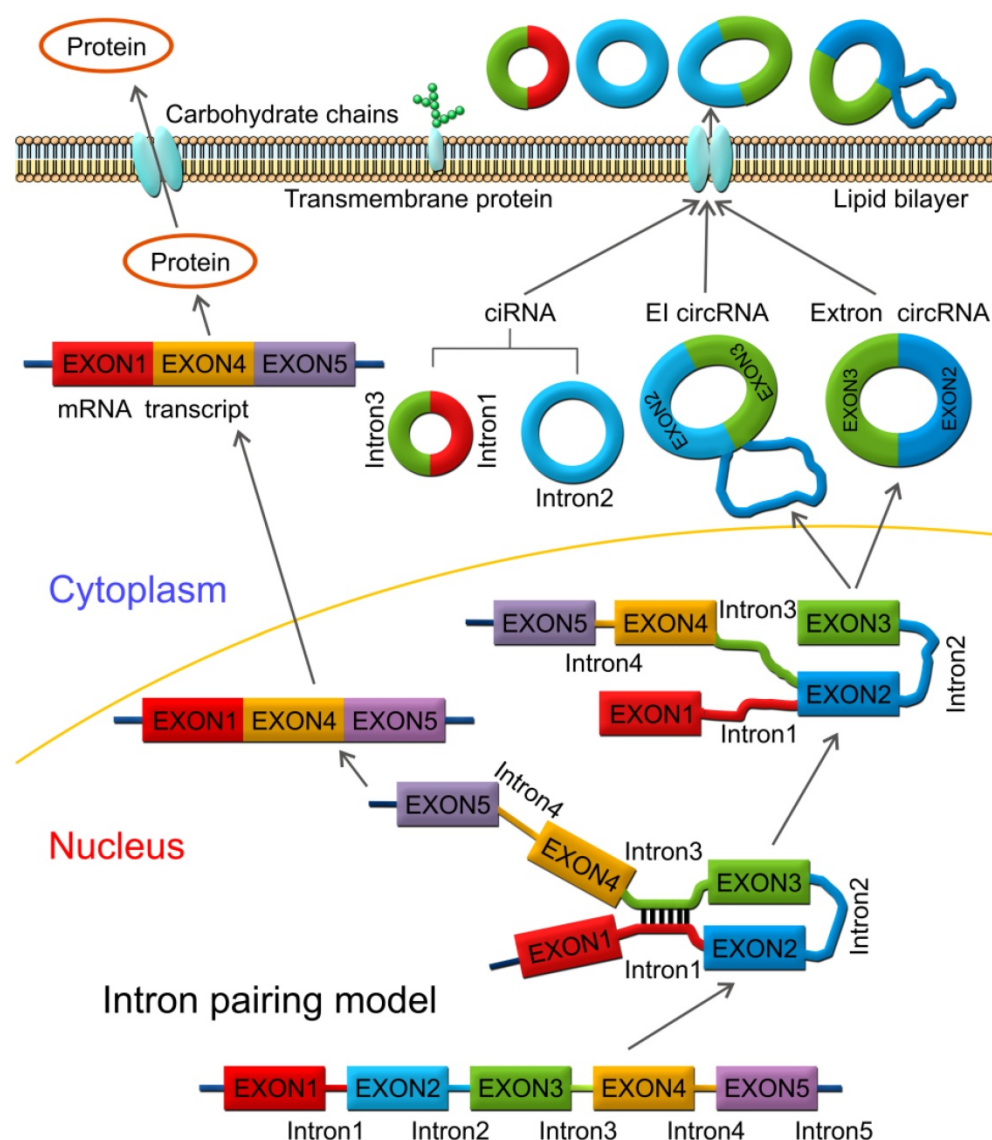


Figure 2. Direct back splicing model. The unspliced introns 1 and 3 interact through complementary base pairing. CircRNAs are formed and released by circularization of exon 2 - exon 3, or exon 2 - intron 2 - exon 3. CircRNAs are present in blood, bodily fluids, and tissues and may serve as biomarkers for human cancers, such as the has_circ_0029 in gastric cancer; Has_circ_100855 in laryngeal squamous cell cancer; circ_CDYL and has_circ_001569 in colorectal cancer; has_circ_0005075 in hepatocellular carcinoma etc.

RBPs are key components in the circularization of RNAs. As shown in Figure 3, base pairing is mediated by RBP between intron 1 and intron 3. Thus, a circular structure is formed and the introns are removed to form circRNAs [26, 70]. The formation of circRNAs has also been shown to be affected by RBPs, which bind to the target sites in the flanking introns and induce circularization and biogenesis of circRNA (Figure 3). Two such examples are of RBPs, such as muscleblind (MBNL1) and adenosine deaminase 1 acting on RNA (ADAR1), which have been reported to enhance or suppress circRNA formation and biogenesis by different modes of action. MBNL1 binds to its own pre-mRNA and bridges two flanking introns close together to induce back-splicing [59]. ADAR1 regulation is involved in Adenosine-to-Inosine (A-to-I) editing and regulates circRNA biogenesis directly through its dsRNA binding activity [71]. Intronic circRNAs are formed by intron-driven circularization model [73-75].

During the intron-driven circularization, lariat introns are processed from primary transcripts in a

two-step mechanism. First, the 2'-OH group of the branch point adenosine (bpA) attacks the 5'-splice site generating a 3'-OH group at the 5'-exon. Next, the newly generated 3'-OH group carries out a nucleophilic attack on the 3'-splice site forming an excised lariat intron and a linear RNA transcript composed of the alternative combined exons (Figure 4) [76].

CircRNAs in diagnosis and prognosis of human cancers

CircRNAs have been reported to play important roles in various diseases, such as atherosclerosis [77], nervous system disorders [78], diabetes [79], and cancer [80, 81]. The unique features and diverse functions of circRNAs make them promising candidates with clinical potentials in life sciences and medicine. Here, we summarize the role of circRNAs in cancer with specific reference to cellular development, proliferation, and apoptosis.

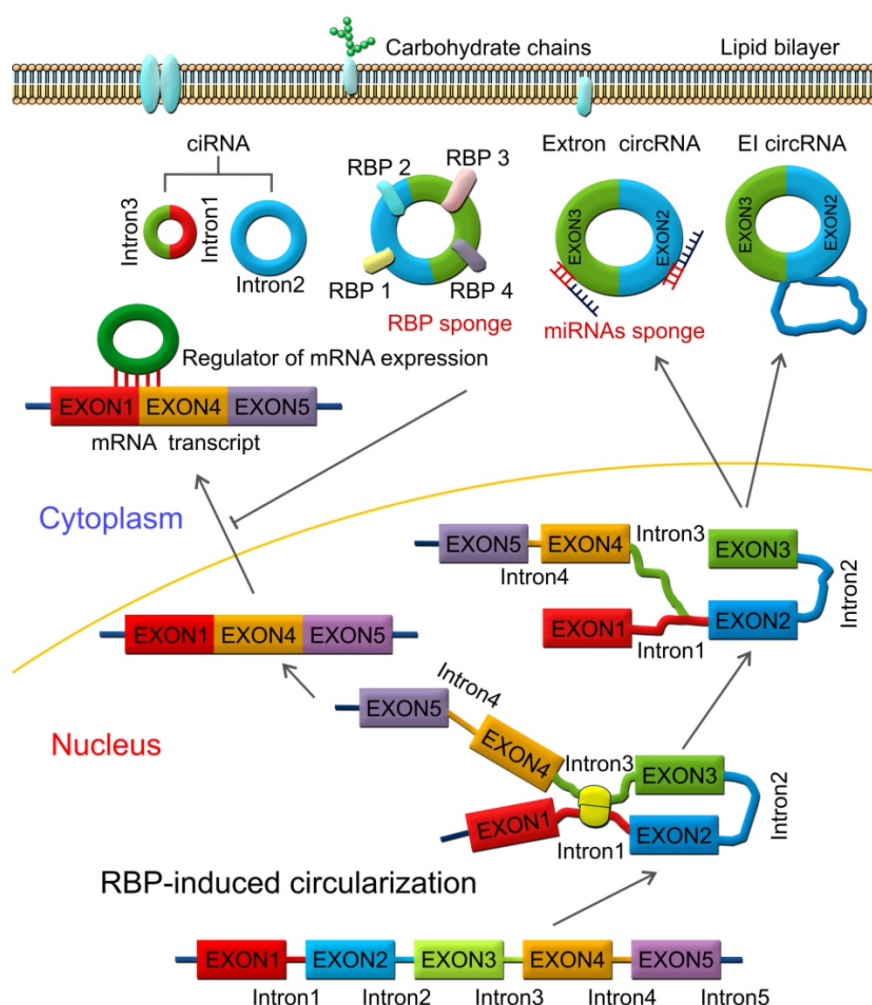


Figure 3. RNA-binding protein (RBP)-induced circularization model. Base-pairing is mediated by RBP between intron 1 and intron 3. Circular structure is formed and the introns are then removed to form circRNAs, which can function as miRNA or RBP sponges and regulators of mRNA expression.

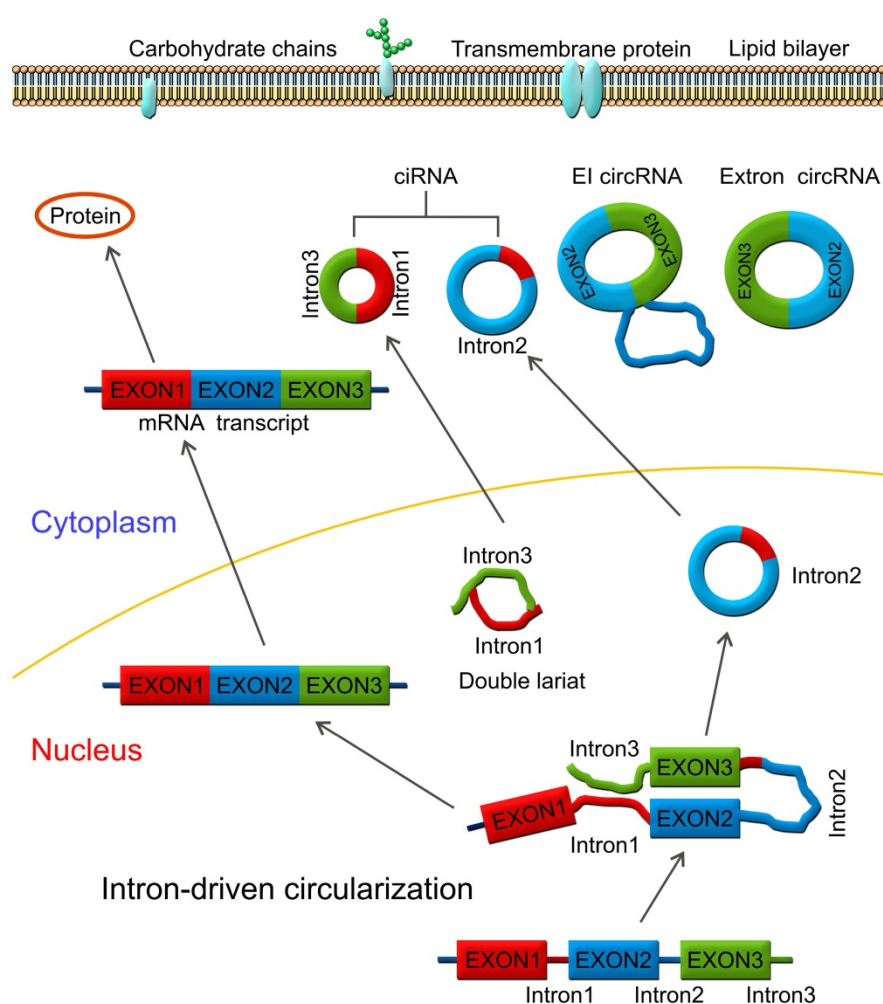


Figure 4. Intron-driven circularization model. Intronic circRNAs are generated via a two-step mechanism. The 2'-OH group of a defined adenosine within intron 2 attacks the 5'-exon and the lariat intermediate is formed as well as a free 3'-OH group at the 5'-exon. Next, the generated 3'-OH group attacks the 3'-splice site to produce an excised lariat intron and a linear RNA product.

CircRNAs are differentially expressed in radioresistant cancer cells

Complex multidisciplinary methods incorporating surgery, chemotherapy, radiotherapy, and immunotherapy are applied for the treatment of human cancers and the molecular alterations that ensue following these treatments have been amply studied. However, the expression profiles of circRNAs in various therapeutic models have not been widely studied. Differential expression of circRNAs in radioresistant cancer cells has been reported [82]. Radiotherapy is one of the most important modalities for cancer treatment [82, 83]. Acquired radio-resistance during the course of the treatment is considered to be the main reason for the failure of radiation therapy and tumor recurrence [84-86]. CircRNAs, functioning as miRNA sponges, regulate the expression of numerous cancer-related miRNAs and are believed to be involved in the progression of human cancers [87]. The

circRNA-miRNA-mRNA axis has been reported to play a role in several cancer-associated pathways with both agonist or antagonist effects on carcinogenesis and metastasis [88]. In this study, a comprehensive expression and functional profile of differentially expressed circRNAs in radioresistant esophageal cancer cells was determined which indicated the possible involvement of the dysregulated circRNAs in the development of radiation resistance. Among the detected 3752 circRNA candidates, 57 circRNAs were significantly upregulated and 17 circRNAs were downregulated in human radioresistant esophageal cancer cell line KYSE-150R compared to the parental cell line KYSE-150 (fold change ≥ 2.0 and $P < 0.05$). Furthermore, GO bioinformatics analysis showed the enrichment of 400 target genes including most miRNAs in the Wnt signaling pathway. CircRNAs_001059 and _000167 were the two largest nodes in circRNA/miRNA co-expression network [82].

CircRNAs are involved in signaling pathways in human cancers

CircRNAs are relatively stable in animal cells and can regulate the target gene expression in several ways. First, circRNAs, have a covalently closed loop with no 5'-3' polarity or poly A tail; the circular loop consists of various miRNA binding sites known as miRNA response elements (MREs) enabling circRNAs to act as miRNA sponges. Second, circRNAs can also regulate mRNA expression by functioning as RBP sponges. And third, circRNAs can regulate mRNA expression by directly binding to targeted mRNAs. By virtue of their interactions with miRNA, circRNAs play key roles in regulating cancer progression and may be involved in a variety of signaling pathways in cancers [89-93] (Figure 5) such as mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK1/2), phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) intracellular signaling pathway, and Wnt/beta-catenin pathway [94-99] (Table 2). Here, we present a brief overview of numerous examples of specific and mainly cancer-related circRNAs that have the potential of directly or indirectly impacting key signaling pathways and cellular processes.

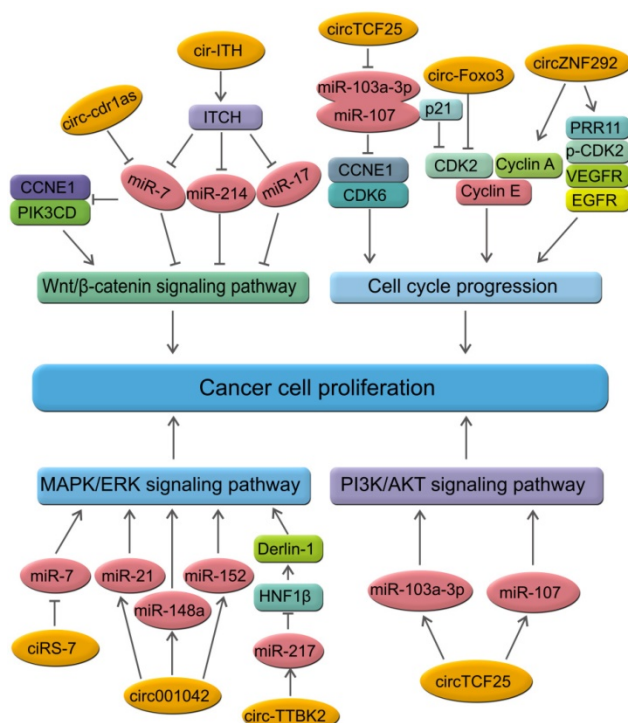


Figure 5. CircRNAs and the related signaling pathways in human cancers. Wnt/ β -catenin signaling pathway plays an important role in the progression of human cancers. Cir-ITCH and circ-cdr1as are two key circRNAs that regulate the proteins or miRNAs in Wnt/ β -catenin signaling pathway. CircTCF25, circ-Foxo3 and circZNF292 are three key circRNAs that regulate cell cycle progression in various cancers. MAPK/ERK and PI3K/AKT are key signaling pathways involved in human cancers. Several circRNAs such as circS-7, circ001042, circ-TTBK2 and circTCF25 have been identified as regulators of these signaling networks.

Several circRNAs have been reported to impact the EGFR/RAF1/MAPK signaling. Activation of the EGFR/RAF1/MAPK pathway has been reported via suppression of miR-7 activity. The circRNA CiRS-7 was proposed to be a potential miR-7 sponge thus impacting the EGFR/RAF1/MAPK [100]. MRPS35_hsa_circ_001042 was predicted to interact simultaneously with miR-21, miR-148a, miR-148b, and miR-152 in the MAPK signaling pathway [101]. In osteoarthritis (OA), circRNA-CER was reported to function as a sponge by competitively binding miR-136 and could target and regulate MMP13 through TGF β , JNK, and ERK pathways [102]. Circ-TTBK2 was upregulated in glioma tissues and cell lines and exerted an oncogenic role in glioma cells. Inhibiting circ-TTBK2 restrained malignant progression of glioma cells by upregulating miR-217, which exerted tumor-suppressive function through downregulating HNF1 β , which, in turn, activated Derlin-1 via binding to its promoter and activated PI3K/AKT and ERK signaling pathways [103]. Thus, inhibition of circ-TTBK2/miR-217/HNF1 β /Derlin-1 axis may be a potential therapeutic target for human gliomas. Another example is of miR-21/ERK signaling which was reported to be a potential therapeutic pathway for the prevention of myocardial fibrosis [104]. Upregulation of miR-21 and miR-200 blocked hsa-circ-000595 by regulating cell apoptosis [105]. Another example is of circTCF25-miR-103a-3p/miR-107 axis which participates in many significant pathways in cancer. Significantly, the PI3K-AKT signaling pathway overlaps with the miR-103a-3p and miR-107 axis [83].

Circular RNA-ITCH (cir-ITCH) was significantly decreased in lung cancer tissues compared to the paired adjacent noncancer tissues obtained from 78 patients. Ectopic expression of cir-ITCH markedly enhanced the level of its parental cancer-suppressive gene, ITCH, and inhibited the cell viability of lung cancer cells by targeting oncogenic miR-7 and miR-214. Furthermore, cir-ITCH acted as a sponge of oncogenic miRNAs and suppressed Wnt/ β -catenin signaling [106]. This was also confirmed in esophageal squamous cell carcinoma (ESCC) in which cir-ITCH acted as a sponge for miR-7, miR-17, and miR-214. Increased expression of ITCH by cir-ITCH, in turn, enhanced the ubiquitination and degradation of phosphorylated Dvl2 (Segment polarity protein disheveled homolog DVL-2), thereby inhibiting the Wnt/ β -catenin pathway. These data revealed that cir-ITCH has an inhibitory effect on ESCC via its effect on the Wnt pathway [107]. In another study of 45 pairs of specimens from colorectal cancer (CRC) patients, cir-ITCH expression was significantly down-regulated in CRC tissues compared to the

peritumoral tissue further confirming that cir-ITCH was involved in the inhibition of the Wnt/ β -catenin pathway [108].

Chen *et al.* compared the differentially expressed circRNAs in gastric cancer (GC) and paired normal tissues and identified at least 5500 distinct circRNA candidates. CircPVT1, derived from the PVT1 gene, was upregulated in GC tissues and promoted tumor proliferation by acting as a sponge for members of the miR-125 family. CircPVT1 could serve as an independent prognostic marker for overall survival and disease-free survival in patients with GC [109]. CiRS-7, a potential miR-7 sponge, was significantly up-regulated in colorectal cancer tissues compared with matched normal mucosae and emerged as an independent risk factor for overall survival. Thus, CiRS-7 was a promising prognostic biomarker and served as a therapeutic target for reducing EGFR-RAF1 activity in colorectal cancer patients [100].

CircRNA sponges withstand enzymatic degradation and are completely resistant to miRNA-mediated degradation. Studies showed that circRNAs against miR-21 or miR-22 are more effective than the typical linear miRNA sponges and the miRNA inhibitors in repressing miRNA targets. Some circRNAs also display superior anti-cancer activities compared to the linear sponges as shown in malignant cell lines [110, 111]. Especially, the expression levels of circRNAs and linear RNAs showed an inverse trend for many cancer-related signaling pathways including NF κ B, PI3k/AKT, and TGF- β [112-115].

Circular RNA Cdr1as expression was upregulated in hepatocellular carcinoma tissues compared to the adjacent non-tumor tissues. Knockdown of Cdr1as or overexpression of miR-7 suppressed HCC proliferation and invasion by inhibiting the CCNE1 and PIK3CD expression. Importantly, knockdown of Cdr1as suppressed the HCC cell proliferation and invasion through targeting miR-7. Thus, Cdr1as plays an important role to promote HCC progression as an oncogene partly through targeting miR-7 [116]. This was contrary to the myocardial infarction mouse model in which Cdr1as and miR-7a were both upregulated and Cdr1as overexpression in myocardial cardiomyocytes promoted cell apoptosis but was reversed by miR-7a overexpression [117]. This study implied the roles of circRNAs were tissue specific and the circRNA-miRNA association was bidirectional.

It has been reported that cZNF292 is a new circular oncogenic RNA involved in the progression of tube formation. Knockdown of cZNF292 significantly inhibited the proliferation and cell cycle

progression in glioma cells by suppressing tube formation. Cell cycle progression in human glioma U87MG and U251 cells was halted at S/G2/M phase via the Wnt/ β -catenin signaling pathway and the levels of related genes, such as PRR11, Cyclin A, p-CDK2, VEGFR-1/2, p-VEGFR-1/2, and EGFR were altered suggesting that cZNF292 silencing plays an important role in the tube formation process. Cznf292 could, therefore, be used as a therapeutic target and biomarker in gliomas [118].

In bladder carcinoma tissues, circRNA expression profiles were screened by microarray analysis. The results demonstrated that in a total of 469 dysregulated circular transcripts, 285 circRNAs were up-regulated and 184 were down-regulated in bladder cancer tissues compared to normal tissues. Over-expression of circTCF25 could sequester and down-regulate miR-103a-3p/miR-107 potentially leading to the up-regulation of thirteen targets including CDK6 which promoted cell proliferation, migration, and invasion *in vitro* and *in vivo* [83].

Another example is of the forkhead family of transcription factor 3 (Foxo3) circRNA, which binds to eight miRNAs to regulate Foxo3. Ectopic expression of Foxo3 circRNA inhibited tumor growth, cancer cell proliferation, and survival by suppressing angiogenesis [119]. It has been reported that circ-Foxo3 was highly expressed in normal cells and was associated with cell cycle progression. Knockdown of endogenous circ-Foxo3 promoted cell growth, whereas ectopic expression of circ-Foxo3 could bind to the cell cycle proteins cyclin-dependent kinase 2 (also known as cell division protein kinase 2 or CDK2) and cyclin-dependent kinase inhibitor 1 (or p21) to form a circ-Foxo3-p21-CDK2 ternary complex blocking cell cycle progression [120].

The expression patterns of circRNAs in the mammary glands of lactating rats were analyzed. Numerous circRNAs were differentially and specifically expressed at different lactation stages. At two different lactation stages in rat mammary glands, a total of 6,824 and 4,523 circRNAs were identified. Also, 1,314 circRNAs were detected at both lactation stages. An interesting observation was that four protein-coding genes (Rev3l, IGSF11, MAML2, and LPP) transcribed high levels of circRNAs involved in breast development and breast cancer [121]. Expression profiling and the comparative analysis of 46 gliomas and normal brain samples detected thousands of circRNAs, 476 of which were differentially expressed [122].

A variety of circRNAs were identified which were potentially involved in the tumorigenesis of basal cell carcinoma (BCC). Twenty-three upregulated and 48 downregulated circRNAs with 354 MREs were

reported which were capable of sequestering miRNA target sequences of the BCC miRNome [123]. Additionally, 322 circRNAs were identified as differentially expressed in cutaneous squamous cell carcinoma (cSCC). Among these, 143 circRNAs were upregulated and 179 were down-regulated. Furthermore, a total of 1603 MREs were found to be differentially expressed in cSCC participating in the epigenetic control [124].

An abundant circRNA, circHIPK3, derived from exon2 of the HIPK3 gene, was observed to sponge nine miRNAs with 18 potential binding sites. It was reported that circHIPK3 directly bound to miR-124 and inhibited miR-124 activity. Knockdown of circHIPK3 but not HIPK3 mRNA could significantly

inhibit human cancer cellular growth by sponging multiple miRNAs [27].

CircRNAs are potential novel biomarkers for diagnosis and prognosis of human cancers

CircRNAs have been shown to regulate gene expression through various mechanisms and can be useful biomarkers in a variety of diseases due to their stability and specific expression. Also, circRNAs can serve as oncogenic stimuli or tumor suppressors in cancer and are therefore of potential significance in cancer prognosis and treatment (Table 1) [22, 125, 126]. Here, we summarize several circRNAs, which were used as novel biomarkers in the prognosis and clinical therapy of various human cancers.

Table 1. CircRNAs as diagnostic and prognostic markers for human cancers

circRNAs	Cancer	Function	Reference
Hsa_circ_0001649	hepatocellular carcinoma	Regulates tumorigenesis and metastasis and might serve as a novel potential biomarker for HCC.	[129]
ciRS-7 (Cdr1as)	hepatocellular carcinoma	May be a promising biomarker of hepatic microvascular invasion (MVI) and a novel therapeutic target for restraining MVI in hepatocellular carcinoma.	[116, 130, 135]
hsa_circRNA_100855 and hsa_circRNA_104912	laryngeal squamous cell cancer	hsa_circRNA_100855 was the most upregulated circRNA and hsa_circRNA_104912 was the most downregulated circRNA in LSCC. Patients with T3-4 stage, neck nodal metastasis, poor differentiation or advanced clinical stage had a higher level of hsa_circRNA_100855 and lower hsa_circRNA_104912 expression.	[134]
Hsa_circ_002059	Gastric cancer	Significantly downregulated in gastric cancer tissues compared with paired adjacent nontumorous tissue and had a significant correlation with distal metastasis (P=0.036), TNM stage (P=0.042), gender (P=0.002), and age (P=0.022).	[136]
Hsa_circ_001988	Colorectal cancer	Decreased expression was observed in colorectal cancer. Can serve as a novel potential biomarker for diagnosis and a potential novel therapeutic target for colorectal cancer	[142]
circTCF25	Human bladder cancer	Could sequester miR-103a-3p/miR-107 potentially leading to the up-regulation of 13 targets related to cell proliferation, migration, and invasion and thus serve as a new promising marker for bladder cancer.	[83]

Table 2. CircRNAs and the related signaling pathways in human cancers

circRNAs	MiRNA target	m RNA-miRNA target	Cancers	Signaling pathway	reference
circRNA_001059 and circRNA_000167	/	/	Radioresistant esophageal cancer	Wnt signaling pathway	[82]
Cir-ITCH	miR-7, miR-17 and miR-214	ITCH	Lung cancer	Cir-ITCH inhibits lung cancer progression by enhancing its parental gene, ITCH, expression and suppresses the activation of Wnt/beta-catenin signaling.	[106-108]
Cdr1as	miR-7	CCNE1 and PIK3CD	hepatocellular carcinoma	Knockdown of Cdr1as inhibited HCC cell proliferation and invasion by targeting miR-7 and Cdr1as and functioned as an oncogene partly through targeting miR-7 in HCC.	[116]
cZNF292	/	PRR11, Cyclin A, p-CDK2, VEGFR-1/2, p-VEGFR-1/2 and EGFR	human glioma U87MG and U251 cells	cZNF292 is an important oncogenic circRNA. Knockdown of cZNF292 significantly inhibited proliferation of glioma cells and cell cycle progression by suppressing tube formation and could be a therapeutic target and biomarker in glioma.	[118]
CircTCF25	MiR-103a-3p/miR-107	CDK6	Bladder cancer	Over-expression of circTCF25 down-regulated miR-103a-3p and miR-107, increased CDK6 expression, and promoted proliferation and migration <i>in vitro</i> and <i>in vivo</i>	[83]
Foxo3 circRNA	Eight miRNAs, including miR-103a-3p/miR-107	FOXO3, CDK6, CDK2, p21, CCNE1	Mouse cancer cells lines (NIH3T3, 4T1 and B16)	Ectopic expression of Foxo3 circRNA inhibited tumor growth, cancer cell proliferation, and survival by suppressing angiogenesis and cell cycle progression.	[119, 120]
circHIPK3	miR-124		Human cells	circHIPK3 binds to miR-124 and inhibits its activity. Knockdown of circHIPK3 significantly suppressed human cancer	[27]

Serum exosomal circRNAs have been used to distinguish patients with colon cancer from healthy controls [127]. More than 1,800 circular RNAs were analyzed. The ratio of circular to linear RNA isoforms was significantly lower in tumors compared to normal colon samples and much lower in colorectal cancer cell lines and correlated negatively with the proliferation index [128]. Using 89 paired specimens of HCC and adjacent liver tissues, hsa_circ_0001649 was found to be significantly downregulated and its level of expression was closely correlated with tumor size ($p = 0.045$) and the occurrence of tumor embolus ($p = 0.017$). The study concluded that hsa_circ_0001649 was involved in the regulation of tumorigenesis and metastasis of HCC and might serve as a novel potential biomarker for human HCC [129]. Another example is of CiRS-7 (also termed as Cdr1as), which is the inhibitor and sponge of miR-7 in the embryonic zebrafish midbrain and islet cells. Compared with the matched non-tumor tissues, the ciRS-7 level was significantly decreased in 60.2 % specimens of HCC tissues (65 out of 108, 60.2 %). However, high expression of ciRS-7 in HCC tissues was tightly associated with hepatic microvascular invasion (MVI), AFP level, and young age and thus partly related to the progression of HCC. Furthermore, the level of ciRS-7 in HCC tissues with concurrent MVI was inversely associated with that of miR-7. Also, ciRS-7 level was positively associated with the expression of two miR-7-targeted genes, PIK3CD ($r = 0.55$, $p < 0.001$) and p70S6K ($r = 0.34$, $p = 0.021$). Thus, ciRS-7 was one of the independent factors of hepatic MVI [130]. In addition, the levels of hsa_circ_0000520, hsa_circ_0005075, and hsa_circ_0066444 were shown to be differentially expressed in HCC tissues ($n = 3$) and adjacent normal liver ($n = 3$) tissues. The expression level of hsa_circ_0005075 was significantly higher in 60 HCC tissues than the matched nontumorous tissues, was correlated with HCC tumor size, and showed good diagnostic potential. Furthermore, hsa_circ_0005075 targeted the miRNA genes, including miR-23b-5p, miR-93-3p, miR-581, miR-23a-5p, and their corresponding mRNAs, suggesting hsa_circ_0005075 could participate in cell adhesion during HCC development [131]. The expression of miR-23b and miR-23a was associated with cancer metastasis and invasion. For example, miR-23b regulated cell migration and invasion of glioblastoma cells via targeting of Pyk2 [132]; MiR-23a modulated the migration and invasion of non-small cell lung cancer cells by targeting insulin receptor substrate-1 (IRS-1) [133].

CircRNAs were studied in 4 paired laryngeal squamous cell cancer tissues (LSCC) and adjacent

non-tumor tissues by microarray analysis. The results showed that 302 circRNAs were significantly increased and 396 circRNAs were decreased in LSCC tissues; hsa_circRNA_100855 was the most upregulated circRNA and hsa_circRNA_104912 was the most downregulated circRNA. Altered levels of these two circRNAs were associated with the T3-4 stage, neck nodal metastasis or advanced clinical stage, implying their important roles in the tumorigenesis and the potential to serve as novel and stable biomarkers for the diagnosis/prognosis of LSCC [134]. The ciRS-7, the miRNA sponge for miR-7 and sex-determining region Y (SRY), regulated the biological behavior of various cancers by targeting miRNA-7 (miR-7) and miRNA-138 (miR-138) [135]. In gastric cancer patients, hsa_circ_002059 levels were significantly decreased in cancer tissues compared with paired adjacent nontumorous tissues, as well as in the plasma between preoperative and postoperative gastric cancer patients. Furthermore, lower levels of hsa_circ_002059 were significantly correlated with distal metastasis, TNM stage, gender, and age, indicating hsa_circ_002059 could be used as a potential novel and stable biomarker to screen gastric carcinoma [136].

In the clinical setting, circRNAs, which are found in blood, bodily fluids or tissues as a sign of human cancers, are used as biomarkers to detect human cancers (Figure 2). The stability, sensitivity, and specificity of circRNAs must be suitable and appropriate to be used as a biomarker, such as the has_circ_0029 in gastric cancer, has_circ_100855 in laryngeal squamous cell cancer, circ_CDYL and has_circ_001569 in colorectal cancer, and has_circ_0005075 in hepatocellular carcinoma. Other new circRNAs that were recently discovered might also serve as possible biomarkers.

Perspectives and future directions

With the rapid advances in RNA sequencing and analysis, thousands of endogenous circRNAs have been detected in mammalian cells which are highly conserved [137, 138]. So far, the identification and function of circRNAs in human cancers remained elusive. In this review, we described the wide-spread expression of circRNAs as well as their functions. We also summarized our current understanding of their molecular regulatory mechanisms including involvement in crucial signaling pathways. However, several outstanding issues that need to be clarified and/or areas of circRNAs research to focus on include the following:

1. Although circRNA transcripts were discovered in the early 1980s, the information on various aspects of circRNAs is still limited [16]. Recently,

circRNAs have emerged as key regulators in the development and progression of multiple human cancers. Furthermore, circRNAs show a more robust expression pattern across patients than mRNAs and are believed to be involved in tumor growth and metastasis, suggesting that circRNAs have a great potential to be employed as biomarkers in clinical diagnosis for a variety of cancers. There is a need to expand the screening in a variety of human cancers and identifying new candidate circRNAs that can serve not only as reliable diagnostic and prognostic biomarkers but also as therapeutic targets [139] [140].

2. The presence of circRNAs for the detection of not only cancers but also a variety of other diseases needs to be explored in easily accessible specimens of blood, saliva, urine, and feces to realize their full clinical potential.
3. CircRNAs are novel non-coding RNAs which appear to function as oncogenes or tumor suppressor genes in human cancers. One of the molecular mechanisms through which some circRNAs regulate cancer progression is by targeting specific miRNAs [116, 141]. The role of circRNAs in cellular transcriptional control needs to be further explored with respect to cancer development.
4. CircRNAs with multiple MREs are highly stable and effective inhibitors [123, 124]. It would be important to explore whether the sequence and number of MREs can be manipulated to enhance the regulatory effects of circRNAs for the therapy of human cancers.

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Competing Interests

The authors have declared that no competing interest exists.

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