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CircRNA in cancer: Fundamental mechanism and clinical potential

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ABSTRACT

Circular RNAs (CircRNAs) are a class of single-stranded noncoding RNAs that are formed in a circular conformation via non-canonical splicing or back-splicing events. Aberrant expressions of many circRNAs are observed in diverse cancers, indicating their crucial roles in tumorigenesis and tumor development. Recently, several pieces of evidence have revealed that many circRNAs are involved in the promotion or suppression of cancers to varying degrees via different molecular mechanisms. Here in this review, we present a summary of the characteristics, types, biogenesis, and functions of circRNAs, and outline a series of the most recently studied circRNAs and their functional mechanisms in multiple cancer types with future perspectives. With great advances in nucleic acid-based therapeutic tools, circRNAs could be further explored as targetable molecules in future cancer treatments.0

1. Introduction

1.1. The exploration of circRNA

Circular RNA (circRNA) is a special type of RNA with a covalently-closed continuous loop in most cases through 5′ to 3’ ends via back-splicing in animals [1]. More than four decades ago, circular RNAs were first found in plant viroids, which were covalently closed circular RNA molecules [2]. In 1979, electron microscopic images first revealed RNAs with circular conformation in several RNA viruses under partially denaturing conditions [3], followed by the discovery of the first circular RNA in hepatitis delta virus (HDV) in 1986 [4]. Despite the biological significance, the circular structure of single-stranded RNA was still unexplored at that time. It was speculated that these “pan-handles” at the ends of RNA molecules may serve as the initiation site for viral RNA replication [2,3]. For the next several years after the first discovery, a few more circularized RNAs were found in eukaryotes [5–9], however, with little explanation for their possible biological functions.

In 2010 and 2011, circular forms of RNAs corresponding to linear noncoding RNAs (ncRNAs), as well as antisense RNAs, were discovered with clinical implications [10,11], which suggested a possibility that these circularized forms of RNA might correlate with certain diseases. CircRNAs were long considered as by-products of low abundance from aberrant RNA splicing or noise in the genomes [12]. Great advances of high-throughput sequencing technology and novel bioinformatics algorithms have now enabled researchers to discover more types of circRNAs in multiple model organisms and thus to investigate their potential roles in various diseases [13]. In 2012, Salzman et al. first proposed that a significant portion of known noncoding RNAs were with circular forms, and these circRNAs were not simply by-products of RNA splicing [14]. Afterwards, Jeck et al. identified more than 25,000 circRNA species from exon(s) of protein-coding genes, which could also be reproducibly enriched by RNase R digestion [15]. Notably, circRNAs lack 5′ or 3′ ends, which make them inaccessible to RNase R, an RNA endonuclease that degrades all linear RNAs with short 3’ tails but does not degrade lariat or circular forms. To date, RNase R digestion remains the gold standard for the verification and enrichment of circRNAs. Jeck et al. also demonstrated that exon circular RNAs (ecircRNAs) were abundant, stable, conserved, and nonrandom products of RNA splicing that possibly functioned in the regulation of genes [15]. Memczak et al. furthermore confirmed that circRNAs were present in various animal genomes in a complex tissue-, cell-type- or developmental-stage-specific manner [16]. Mechanistically, they proposed lines of evidence that at least certain circRNAs could act as post-transcriptional regulators through competing with mRNAs for binding with considerable microRNAs (miRNAs) and/or RNA-binding proteins (RBPs). The above three

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findings pulled back the curtain on circRNA biology and greatly inspired researchers to engage in discovering novel forms of circRNAs and their potential functions in multiple contexts.

1.2. Subtypes of circRNAs

To date, there are essentially three subclasses of circRNAs identified according to their distribution and biogenesis (Fig. 1): exonic circRNAs (ecRNAs) which are formed via back-splicing of the 5′ splice site (splice donor site) to a 3’ splice site (splice acceptor site) [17,18], intronic circRNAs (ciRNAs) that are formed from intronic lariat precursors escaping from the debranching step of canonical linear splicing [19], and exon-intron circRNAs (EIciRNAs) which are circularized with the retained intronic sequences between the circularized exons and proved to enhance the expression of their parental genes *in cis* [20]. A recently identified circRNAs mitochondria-encoded circRNAs (mecciRNA), which may be generated via a splicing-independent mechanism, can facilitate the mitochondrial entry of nuclear-encoded proteins by serving as molecular chaperones [21]. Novel biotechnics and bioinformatics strategies would expand our understanding of the complexity of circRNAs in the future.

1.3. CircRNA biogenesis

The mechanisms of circRNA biogenesis remain largely unclear. A common and widely acknowledged mechanism is that circRNAs are generally derived from pre-mRNAs and are subject to the canonical spliceosomal machinery [22]. Notably, circRNA characteristic back-splicing reactions require the covalent linking of the 3′ splice site with the 5’ splice site for circRNA biogenesis, which is believed to be facilitated by reverse complementary *Alu* repeats flanking the circularized exon [23]. Some other bioinformatics analyses have also confirmed the association of flanking long introns and reverse complementary *Alu* repeats to the biogenesis of mammalian circRNA [15,20,24,25]. Also, exonic sequences involved in backsplicing might play critical roles for some circRNAs [20,25,26], highlighting the significance of both exonic sequences and intronic repeats in the biogenesis of circRNAs [25]. Some RBPs such as QKI, ADAR1, DHX9, FUS, HNRNPL are also involved in at least certain circRNA biogenesis [27–31]. There is still limited evidence for circRNA export as circRNAs are synthesized in the nucleus but are found to be mostly localized in the cytoplasm [30]. Huang et al. found that circRNA localization is actively controlled by Hel25E homologs in both Drosophila and human cells in a length-dependent manner, providing new insights into nuclear export mechanisms of circRNAs [32].

1.4. Functional mechanisms of circRNAs

1.4.1. miRNA sponge

CircRNAs participate in a variety of biological processes due to their unique structures and other properties [33]. The most prominent circRNA function is the ability to serve as miRNA sponge (Fig. 2A). CircRNAs with miRNA binding sites bind directly to the corresponding miRNAs to inhibit miRNA activity and thus regulate the expression of target genes. The first circRNA serving as a competing endogenous RNA (ceRNA) was CDR1as (antisense to the cerebellar degeneration-related protein 1 transcript), which harbors more than 60 conserved binding sites for miR-7 [16,34]. It bound miR-7 and impaired midbrain development in zebrafish, similar to the knockdown of miR-7. In the same year, Hansen et al. proposed that murine circRNA circSRY served as a miR-138 sponge with more than ten binding sites [34,35]. Despite the discovery of several circRNAs serving as miRNA sponges in recent years [23,36], it is worth noting that these forms just a fraction of the thousands of human circRNAs to harbor multiple computational binding sites for individual microRNAs. Interestingly, *P. falciparum* and *S. cerevisiae* lack miRNA pathway but have abundant circRNAs [37]. Hence, the concept of miRNA sponge might not be a general function for circRNAs.

1.4.2. Transcriptional regulation or splicing

Alternative splicing is widely associated with diverse biological processes. CircRNAs were found to affect alternative splicing through

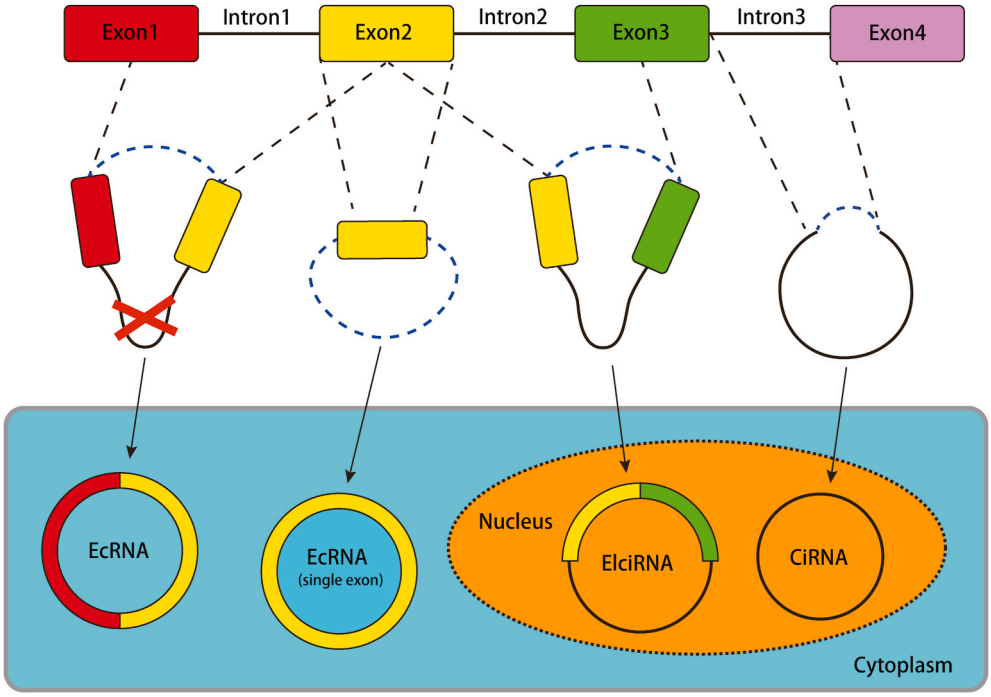

Fig. 1. The biogenesis of circRNAs. Exonic circRNAs (EcRNAs) are formed via back-splicing (blue lines) with multiple exons or single exon, the major forms of circRNAs. Exon-intron circRNAs (EIciRNAs) are circularized with the retained intronic sequences between the circularized exons, predominantly in the nucleus. Intronic circRNAs (ciRNAs) are formed from intronic lariat precursors escaping from the debranching step of canonical linear splicing, abundant in the nucleus.

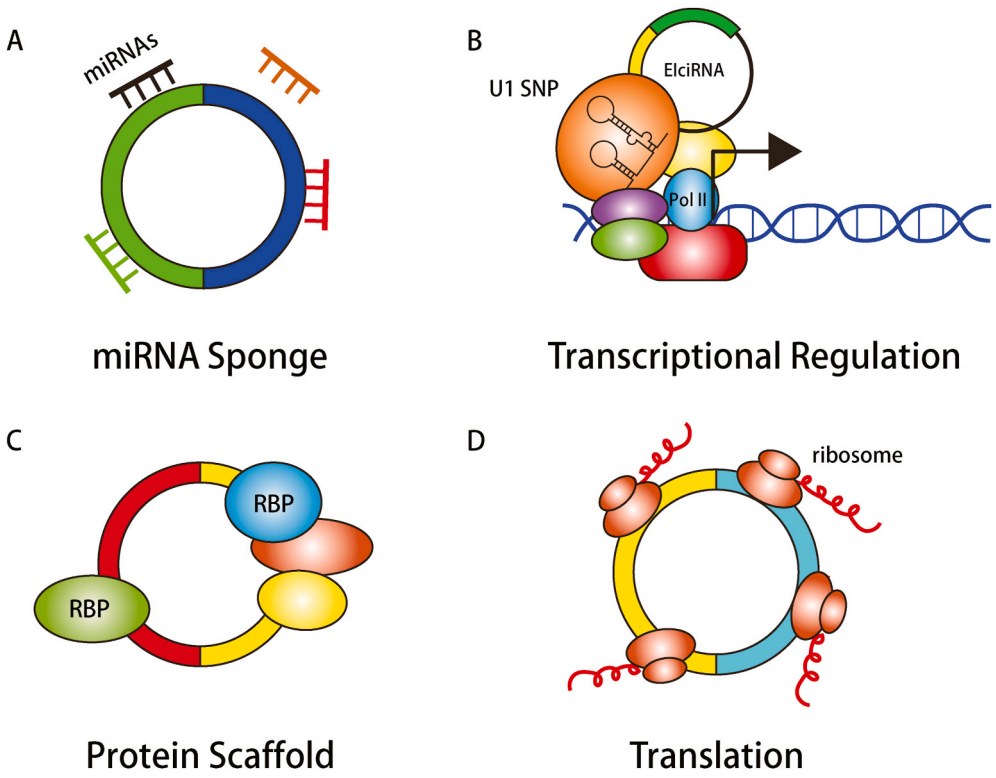

Fig. 2. The functional mechanisms of circRNA. (A) binding to the corresponding miRNAs to inhibit miRNA activity; (B) transcriptional regulation; (C) interacting with RNA-binding proteins as protein scaffolds; (D) translating into proteins.

RNA-mediated interaction, thereby regulating gene expression. Ashwal-Fluss et al. revealed that circMBL, derived from the second exon of the splicing factor muscleblind (MBL/MBNL1), could compete with the linear splicing of pre-mRNA [22]. MBL levels strongly affected circMbl biosynthesis depending on the MBL binding sites. CircMbl could decrease the production of its parental mRNA. Another good case in point was reported in 2015 and suggested that circRNAs could regulate the transcription of their parent genes (Fig. 2B) [20]. Li et al. demonstrated that a subclass of circRNA EIciRNA was predominantly localized in the nucleus and promoted transcription of their parental genes by interacting with U1 snRNP, thereby unveiling a novel regulatory strategy for transcriptional regulation via specific RNA-RNA interactions.

1.4.3. Interaction with RBPs

CircRNAs can also interact with RNA-binding proteins as protein scaffolds or antagonists (Fig. 2C) [27–31]. Du et al. showed that circ-Foxo3 interacted with p21 and CDK2 to form ternary complexes thereby blocking the cell cycle progression [38]. Abdelmohsen et al. demonstrated that circPABPN1 suppressed HuR binding to PABPN1 mRNA, and thus decreased PABPN1 translation [39]. A recent study also provided evidence that N^6-methyladenosine (m^6A) modification of circNSUN2 enhanced the stability of *HMGA2* through m^6A methylation-dependent nuclear export to promote colorectal carcinoma (CRC) metastasis progression [40].

1.4.4. Translation of proteins

Although circRNAs have been considered as "non-coding" elements, the predominantly cytoplasmic distribution of most circRNAs suggests a high possibility of translational potential upon their internal ribosome entry site (IRES) initiation (Fig. 2D). Three consecutive studies in 2017 with convincing pieces of evidence showed some circRNAs could be translated through IRES-driven mechanisms [41–43]. Yang et al. identified hundreds of endogenous translatable circRNAs, many of which contained m^6A sites. This work suggested a possibly common translation pattern for circRNAs [41]. Pamudurti et al. furthermore proved that circMbl generated from the mbl locus could produce a detectable protein, and starvation and FOXO could possibly regulate the translation of a circMbl isoform [42]. Legnini et al. presented circ-ZNF609, with an open reading frame spanning from the start codon could be translated into a protein in a splicing-dependent and cap-independent manner [43]. Despite the controversy and concerns of inefficiency in translation, the translation of hundreds of circRNAs has expanded the coding landscape of human transcriptome.

2. Potential roles of circRNAs in cancers

Recent pieces of evidence revealed that aberrant expressions of circRNAs occur in almost all cancer types and play indispensable roles in cancer pathogenesis, either as oncogenes or tumor suppressors (Fig. 3) [23]. CircRNAs are found to regulate the proliferation, migration, invasion, and apoptosis of cancer cells through various mechanisms [23,44,45]. Since an early diagnosis of cancer patients is particularly critical, the identification and application of effective biomarkers are urgently required. CircRNAs possess unique properties such as tissue- or cell- and developmental stage-specific patterns, resistance to exonucleases and RNase R, and longer half-lives [16]. These unique features suggest that circRNAs could serve as potentially targetable markers in clinical treatments. Below, we will discuss the most recent studies of circRNAs in diverse cancers.

2.1. Gastrointestinal cancer

2.1.1. Hepatocellular carcinoma (HCC)

Hepatocellular carcinoma is the most common type of primary liver cancer, associating with numerous cancer-related deaths [46]. Han et al. demonstrated that circMTO1 (mitochondrial translation optimization 1 homologue; *hsa_circRNA_0007874/hsa_circRNA_104,135*) was down-regulated in HCC and suppressed HCC progression by sponging

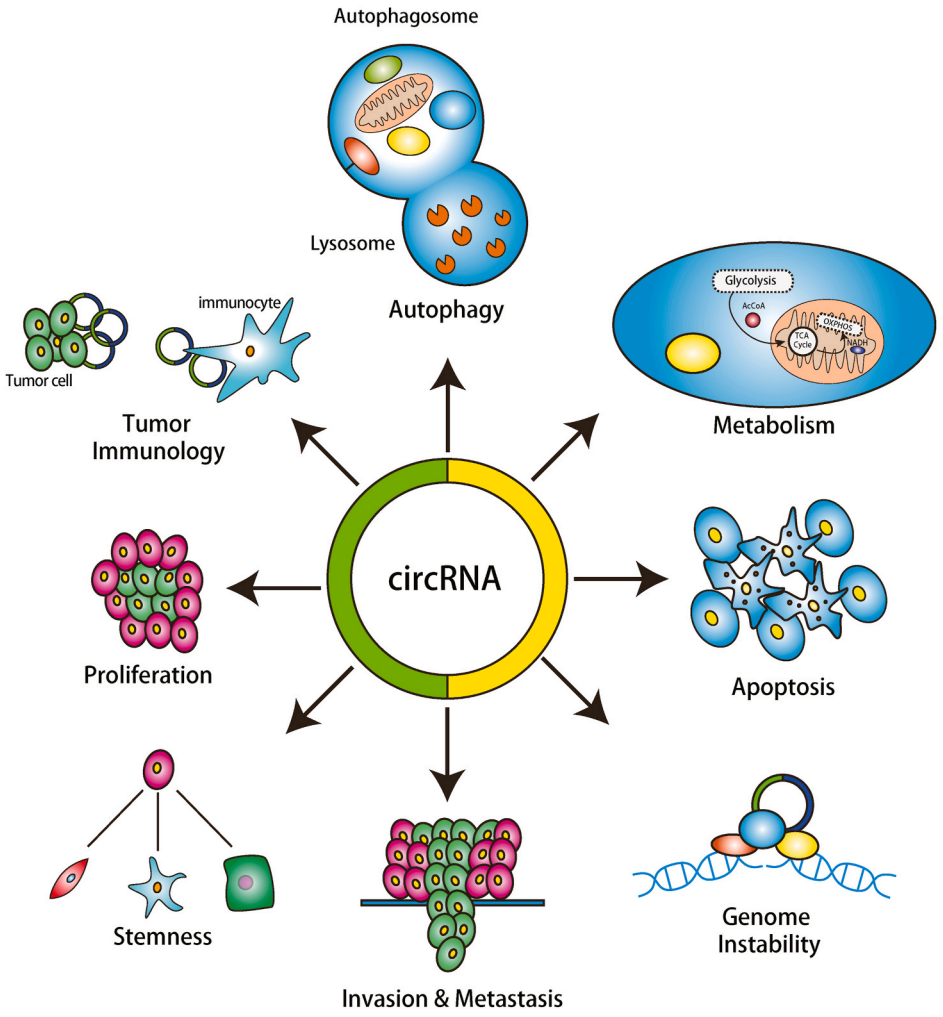

Fig. 3. General description of the circRNA functions in cancers.

oncogenic miR-9 to promote p21 expression [47]. A similar ceRNA mechanism also applies to circTRIM33-12, which was down-regulated in HCC tissues and cell lines. CircTRIM33-12 up-regulated TET1 expression by sponging miR-191, resulting in significantly reduced 5-hydroxymethylcytosine (5hmC) levels in HCC cells [48]. CircTMEM45A sponges miR-665 to relieve its IGF2 target in HCC both *in vitro* and *in vivo* [49]. Meng et al. reported that Twist1 promoted the expression of Cul2 circular RNA (circ-10720) but not mRNA in HCC. Further analyses showed that Twist1 promoted vimentin expression through circ-10720, which could absorb miRNAs that target vimentin [50]. Besides ceRNA network, circRNAs functioning through RBPs were recently reported in HCC. Wang et al. showed that circRHOT1 was significantly up-regulated in HCC, which promoted proliferation and metastasis. Mechanistically, RNA pulldown assay revealed that circRHOT1 recruited TIP60 to the NR2F6 promoter thus initiating NR2F6 transcription [51]. RBM3 is involved in numerous steps of tumor progression [52]. Recently, it was found to dynamically regulate circRNA SCD-circRNA 2 and functioned as an oncogene in an SCD-circRNA 2 dependent manner [53]. CircRNAs are also reported to regulate the stemness of cancer [54]. Zhu et al. recently reported that circZKSCAN1 could competitively bind FMRP to transcriptionally repress Wnt signaling in HCC [55]. Bioinformatics analyses accelerated the study on circRNA and expanded RNA networks in HCC. Sheng et al. investigated HCC samples by RNA-seq and identified a series of dysregulated RNAs in HCC [56]. Functional networks of lncRNA-mRNA, circRNA-miRNA-mRNA were first established in HCC, providing further insights into the gene expression network of HCC.

2.1.2. Colorectal cancer (CRC)

Colorectal carcinoma ranks third in terms of incidence and remains the second leading cause of cancer-related mortality worldwide [57]. Hsiao et al. reported that circCCDC66 was up-regulated in polyps and CRC relating to poor prognosis. Mechanistically, miRNA target sequence analysis revealed that circCCDC66 exons 8 to 10 harbored 99 miRNAs binding sites, implying that circCCDC66 might serve as a miRNA sponge to protect a group of oncogenes from being attacked by a panel of miRNAs [58]. Besides, this work also discussed in depth the complexity of "miRNA sponge" in cancer malignancy and highlighted the significance of circRNA-miRNA regulatory gene network. Han et al. found that circLONP2 was up-regulated in CRC and promoted the maturation of primary microRNA-17 (pri-miR-17), and subsequent intercellular transfer of miR-7. This effect was carried out through DGCR8 and Drosha complex in a DDX1-dependent manner to enhance CRC invasion and metastasis [59]. In 2019, Chen et al. unveiled a novel mechanism that m^6A modification of circNSUN2 increased its export to the cytoplasm through YTHDC1. Further analyses showed that circNSUN2 could stabilize the stability of HMGA2 mRNA to promote CRC metastasis progression via circNSUN2/IGF2BP2/HMGA2 RNA-protein ternary complex, indicating therapeutic implications in CRC [40]. This work provides a piece of strong evidence on the effects of m^6A modification on cellular circRNAs biology.

2.1.3. Gastric cancer (GC)

Gastric cancer is one of the top leading causes of human mortality in malignant tumors, especially in developing countries [57]. For the past

several years, circRNAs were considered as potential "druggable" targets in the clinical treatments of GC. Zhang et al. recently suggested that ciRS-133 was increased in gastric cancer tissues and plasma of gastric cancer patients [60]. Gastric cancer cell-derived exosomes delivered ciRS-133 into pre-adipocytes, where ciRS-133 promoted PRDM16 expression via sponging miR-133. Dai et al. found circFGD4 was down-regulated in human GC tissues and cell lines [61]. Further analysis validated that circFGD4 acted as a sponge for miR-532–3p to relieve the tumor-promoting effects of miR-532–3p on its target APC. Zhang et al. demonstrated that circCACTIN was up-regulated in GC and exerted its function through sponging miRNA-331–3p to regulate TGFBR1 mRNA expression [62]. A similar ceRNA mechanism also applies to circNHSL1, which sponged miR-1306–3p to relieve its repression on SIX1 and thus promoted cell migration and invasion [63]. Several bioinformatics studies have also reinforced that a great number of circRNAs were differentially expressed in GC, more importantly, some of them had important implications on the potential, novel, and non-invasive diagnostic methods for patients with GC [64–67].

2.1.4. Esophageal cancer (EC)

Esophageal cancer (EC) harbors a high incidence rate and mortality worldwide [57]. However, suitable biomarkers with specificity and sensitivity for EC are unsatisfactory and limited. Fan et al. identified 1045 up-regulated and 1032 down-regulated circRNAs from circular RNA microarray, two of which showed potential as blood biomarkers for esophageal squamous cell carcinoma (ESCC), the major type of EC [68]. Shi et al. demonstrated that circLPAR3 was up-regulated in ESCC. CircLPAR3 activated the RAS/MAPK and the PI3K/Akt pathways through sponging miR-198, which promoted the migration, invasion, and metastasis of ESCC cells [69]. Wang identified circ-LRP6, which correlated with malignant clinicopathological characteristics and poor prognosis, sponged miR-182 to relieve the suppression of a proto-oncogene myc, and thereby promoted EC progression [70]. Zhang et al. found that up-regulated hsa_circRNA6448-14 promoted cell proliferation, migration, invasion, and inhibited apoptosis in EC, possibly via competitively binding miR-455–3p [71]. circFNDC3B, circ-SMAD7, has_circ_0067,934, among others were also found to be closely associated with EC progression and might serve as diagnostic and prognostic biomarkers for EC [72–74].

2.1.5. Pancreatic cancer (PC)

The majority of patients with pancreatic cancer have a delayed diagnosis, as an early diagnosis for PC is difficult, resulting in the lowest 5-year relative survival rate (9%) among all cancer types [75]. In 2020, Wong et al. validated that circFOXK2 was significantly up-regulated in pancreatic ductal adenocarcinoma (PDAC) cells and tissues [76]. Evidence showed that circFOXK2 was capable of promoting cell growth, migration, invasion, and apoptosis in complex with circFOXK2-interacting proteins YBX1 and hnRNPK. Guo et al. identified a novel circRNA circBFAR which was up-regulated in a cohort of patients with PDAC [77]. Mechanistically circBFAR functioned by sponging miR-34b-5p to up-regulate mesenchymal-epithelial transition factor (MET) expression. This resulted in the activation of downstream phosphorylation of Akt (Ser 473) and thereby promoting MET/PI3K/Akt signaling pathway. Notably, the administration of a MET inhibitor PHA-665752 (PHA) could compromise circBFAR pro-tumor functions *in vivo*, indicating a therapeutic potential for PDAC therapy [77]. Several other "targetable" circRNAs such as circ-LDLRAD3 and circPDAC were also reported in PC recently [78,79] (Table 1).

Table 1
CircRNAs in gastrointestinal cancers.

Cancer type	circRNA	Expression	Mechanism	Reference
Hepatocellular carcinoma	circMTO1	down-regulated	microRNA sponge	[47]
	circTRIM33-12	down-regulated	microRNA sponge	[48]
	circTMEM45A	up-regulated	microRNA sponge	[49]
	Cul2 circular	up-regulated	microRNA sponge	[50]
	RNA circRHOT1	up-regulated	Interact with RBP	[51]
	SCD-circRNA 2	up-regulated	Interact with RBP	[53]
	circZKSCAN1	down-regulated	Interact with RBP	[55]
Colorectal cancer	circCCDC66	up-regulated	microRNA sponge	[58]
	circLONP2	up-regulated	microRNA sponge	[59]
	circNSUN2	up-regulated	Interact with RBP	[40]
Gastric cancer	ciRS-133	up-regulated	microRNA sponge	[60]
	circFGD4	down-regulated	microRNA sponge	[61]
	circCACTIN	up-regulated	microRNA sponge	[62]
	circNHSL1	up-regulated	microRNA sponge	[63]
Esophageal cancer	circLPAR3	up-regulated	microRNA sponge	[69]
	circ-LRP6	up-regulated	microRNA sponge	[70]
	hsa_circRNA6448-14	up-regulated	microRNA sponge	[71]
Pancreatic cancer	circFOXK2	up-regulated	Interact with RBP	[76]
	circBFAR	up-regulated	microRNA sponge	[77]

2.2. Gynecological cancer

2.2.1. Breast cancer (BC)

Breast cancer is the most frequently diagnosed cancer among women worldwide [57]. Despite recent scientific advances to facilitate early diagnosis and treatments for BC, quite many BC patients still progress to the metastatic stage after therapy, which calls for novel molecules in the study and treatments of BC. Recent pieces of evidence showed that multiple circRNAs were functionally involved in the progression of BC, mostly through miRNA sponging [80,81]. Liu et al. found hsa_circ_001783 was positively correlated with BC, and knockdown of hsa_circ_001783 inhibited the progression of breast cancer cells [82]. Further analysis showed that hsa_circ_001783 could sponge miR-200c-3p to regulate BC proliferation and metastasis. It is widely acknowledged that both circRNAs and autophagy are associated with BC [83,84]. Liang et al. recently identified that an autophagy-associated circRNA circCDYL was up-regulated in BC, and promoted autophagic levels in BC cells via the miR-1275-ATG7/ULK1 axis, indicating a potential prognostic marker for BC [85]. In the treatments of triple-negative breast cancer (TNBC), Yang et al. identified 803 significantly differentially-expressed circRNAs by RNA-seq in the serum exosomes samples from TNBC patients and non-TNBC patients, from which circHSDL2 was found up-regulated in the TNBC cell lines and clinical tissues. CircHSDL2 was further validated to function via sponging let-7a-2-3p in the progression of TNBC. This study provided new insights into the prognostic perspectives for TNBC from serum-derived exosomal circular RNAs [86].

2.2.2. Cervical cancer (CC)

Cervical cancers (majorly CSCCs) rank second among cancer-related deaths in women aged 20–39 [87]. Effective prophylactic vaccines against the most important carcinogenic HPV types are available, but the number of people receiving the vaccine remains low, as well as the 5-year survival rate for advanced CSCC patients. In 2018, Zheng et al. profiled the expression of circRNAs regulated by HPV16 E7 in cervical

cancer cells [88]. Zhao et al. further reported that human papillomaviruses (HPVs) could also generate circRNAs [89]. E7 oncogene derived circRNA circE7 is m^6A modified, cytoplasm localized, and translatable. Disruption of circE7 in CC cell lines led to the decrease of E7 protein level and suppressed CC progression *in vitro* and *in vivo*. Hong et al. demonstrated that circCLK3 was closely related to CC and acted as a ceRNA for miR-320 to abolish its repression of target gene FoxM1, thereby promoting CC progression [90]. Similar ceRNA patterns were also found in the recent CC studies via high throughput sequencing [91], such as hsa_circ_0000515, circRNA_0000285 and hsa_circRNA_0000069 [92–94].

2.2.3. Ovarian cancer (OC)

Ovarian cancer is one of the most aggressive female malignancies with poor prognosis [56]. Recently, Ding et al. found that circ_0072,995 could promote OC cell proliferation, migration, apoptosis, and tumorigenesis *in vivo* by sponging miR-147 to relieve its target CDK6 [95]. Aberrantly expressed oncogenic circRNA hsa_circ_0009910 was identified by Li et al. in OC cells and it was reported to target miR-145 as a microRNA sponge [96]. Wang et al. demonstrated that circEXOC6B suppressed the development of OC cells through up-regulating RSU1 partially via sponging miR-421 [97]. Cisplatin has been a pivotal chemotherapy drug in the treatment of OC, however, cisplatin resistance hinders the prognosis of patients [98]. Zhao et al. demonstrated that conventional circRNA CDR1as could suppress miR-1270 expression as a sponge to reduce cisplatin resistance [99]. Notably, CDR1as expression was detectable in extracted serum exosomes of OC patients, suggesting that this circRNA may serve as a potential biomarker in the future (Table 2).

2.3. Other cancers

2.3.1. Lung cancer

Lung cancers are the most common type of cancers and are attributed to be the leading cause of cancer-related mortality worldwide, with non-small-cell lung cancer (NSCLC) being the largest proportion [56]. Identification of potentially targetable circRNAs in the earliest stages of cancer progression could have important therapeutic significance. Several lines of evidence have long suggested that circRNAs played regulatory roles in the progression and metabolism of lung cancers [100,101]. Steen et al. recently provided a circular RNA landscape of NSCLC from a panel of 60 lung cancer and non-transformed cell lines, showing that substantial subsets of circRNAs correlated with cell proliferation, histological subtype, or genotype [102]. Hong et al. identified a novel exon-derived circRNA circSLC25A16, which could accelerate the glycolysis and proliferation of NSCLC cells [103]. Further investigations validated that circSLC25A16 functioned by interacting with miR-488–3p/HIF-1α, which in turn activated lactate dehydrogenase A (LDHA). Cheng et al. found that circTP63 promoted cell proliferation both *in vitro* and *in vivo* by competitively binding miR-873–3p, which thus relieved the suppression of its target FOXM1 to facilitate cell cycle progression [104]. Qiu et al. presented that proto-oncogenic circPRKCI enhanced proliferation and tumorigenesis of lung adenocarcinoma by sponging both miR-545 and miR-589 and thus abolished their suppression of the oncogenic transcription factor E2F7 [105]. *In vivo* experiments also confirmed this mechanism, indicating therapeutic applications in lung adenocarcinoma.

2.3.2. Bladder cancer (BCa)

Bladder cancer is a common genitourinary carcinoma with high morbidity and mortality rates [56]. Okholm et al. showed that several differentially expressed circRNAs (including circHIPK3 and circCDYL) are associated with non-muscle-invasive bladder cancer (NMIBC) progression [106]. He et al. found that circFUT8 served as miRNA sponge through binding miR-570–3p, leading to increased expression of tumor suppressor gene Krüpple-like factor 10 (KLF10) to inhibit the migration and invasion of BCa both *in vitro* and *in vivo* [107]. Similarly, it was reported that circCASC15 could also act as miR-1224–5p sponge to promote the expression of CREB1 to enhance cell proliferation capability in BCa [108]. More recent studies have also confirmed that multiple circRNAs play critical regulatory roles in the progression of BCa [109,110] (Table 3).

3. Conclusion and perspectives

The past several years have witnessed a dramatic turnover of RNAs with circular forms from “trash” to “treasure”, with multiple lines of evidence unraveling the significant regulatory and physiological functions of circRNAs in biological processes such as tumorigenesis. Currently, much attention has been given to their biogenesis, expression profiles, novel functional mechanisms, and physiological relevance. However, we are still at the dawn of this field as a lot more unknowns await further investigations.

To date, genome-wide bioinformatics analyses have facilitated our understanding, at least in part, of how these circular molecules are generated and degraded in diverse cells, including cancer cells. However, questions are still frequently raised such as: whether circRNAs are generated co-transcriptionally or post-transcriptionally, what mechanisms determine circRNA localization, and what the actual or general molecular mechanisms on circRNA biogenesis and degradation are. Jia et al. identified that an evolutionarily conserved factor GW182, together with its human homologs (TNRC6A, TNRC6B, and TNRC6C), were involved in a specific degradation pathway of circRNAs [111]. This finding strongly indicates precise regulations might exist in the balance of circRNA biogenesis and degradation, which is also an assertive argument against the idea that circRNAs are by-products of splicing, an echoing perspective from early days to even today.

Additionally, this precise regulations also make us wonder to what

Table 2
CircRNAs in gynecological cancer.

Cancer type	circRNA	Expression	Mechanism	Reference
Breast cancer	hsa_circ_001783	up-regulated	microRNA sponge	[82]
	circCDYL	up-regulated	microRNA sponge	[85]
	circHSDL2	up-regulated	microRNA sponge	[86]
Cervical cancer	circE7	up-regulated	translation	[89]
	circCLK3	up-regulated	microRNA sponge	[90]
Ovarian cancer	circ_0072,995	up-regulated	microRNA sponge	[95]
	hsa_circ_0009910	up-regulated	microRNA sponge	[96]
	circEXOC6B	down-regulated	microRNA sponge	[97]
	circRNA CDR1as	down-regulated	microRNA sponge	[99].

Table 3
CircRNAs in other cancers.

Cancer type	circRNA	Expression	Mechanism	Reference
Lung cancer	circSLC25A16	up-regulated	microRNA sponge	[103]
	circTP63	up-regulated	microRNA sponge	[104]
	circPRKCI	up-regulated	microRNA sponge	[105]
Bladder cancer	circFUT8	down-regulated	microRNA sponge	[107]
	circCASC15	up-regulated	microRNA sponge	[108]

extent circRNAs are involved in specific physiological processes such as tumor immunity. It is widely acknowledged that the cancer-related immunity plays a crucial role in tumor progression [112]. CircRNAs are recently reported to extensively participate in antitumor immune regulation and immunotherapy, mostly through miRNA sponging. Deng recently screened differentially expressed circRNAs between T-cell lymphoblastic lymphoma (T-LBL) tissues and normal infantile thymus, and found that circ-LAMP1 was upregulated in cancerous tissues. Mechanistically, circ-LAMP1 sponged miR-615–5p to increase cancer-related protein discoidin domain receptor tyrosine kinase 2 (DDR2) expression [113]. Hsa_circ_0064,428 was found significantly downregulated in HCC patients with high tumor-infiltrating lymphocytes (TILs) through global microarray profiling, and negatively correlated with patient's survival, tumor size and metastasis [114]. Also, circRNAs can serve as vaccine adjuvants or tumor antigens that trigger innate and adaptive immune responses. Chen et al. found transfecting *in vitro* synthesized circRNA to HeLa cells could stimulate innate immunity gene expression, and intracellular sensor retinoic-acid-inducible gene-I (RIG-I) was responsible for immune response to exogenous circRNAs [115]. Furthermore, they also found self and foreign circRNAs had different m^6A modification patterns: exogenous unmodified circRNA adjuvant could induce antigen-specific T and B cell responses through binding to and activating RIG-1 [116]. PD-L1 is closely associated with tumor escape from immune control, thus drugs targeting PD-L1 against multiple cancer types are valuable aproaches for cancer therapy [117, 118]. Hsa_circ_0020,397 served as miR-138 sponge to promote the expression of miR-138 targets TERT and PD-L1 in colorectal cancer [119]. CircFGFR1 sponged miR-381–3p to promote progression and anti-PD-1 resistance in NSCLC, acting as a promising therapeutic target for NSCLC therapy [120]. Exosome-derived hsa-circRNA-002178 sponged miR-34 to enhance PD-L1 expression in lung adenocarcinoma (LUAD) inducing T-cell exhaustion. Notably, circRNA-002178 was found to exist in exosomes of plasma, indicating it could serve as a novel diagnosis biomarker for LUAD [121].

Early, fast, and accurate diagnosis for a patient with cancer is the main priority for clinical observations, as well as clinical cancer research. Circular RNAs have unveiled the mysterious masks of their relevance towards pathology and demonstrated their significant regulatory roles in both prognosis and diagnosis of cancer treatments, based on their fundamental properties. CircRNAs can function through various mechanisms either by promoting or suppressing tumorigenesis. Interestingly, based on the present studies on circRNA functional mechanisms, most pro/anti-tumor functions of circRNAs were miRNA sponges, a concept proposed in 2013 [15,16]. As most of the identified circRNAs were predominantly localized in the cytoplasm, it would be reasonable to speculate that many circRNAs would function as a ceRNA as well. This speculation was then challenged by a line of facts that only several out of thousands of circRNAs harbored computational-predicted miRNA binding sites. Recent evidence also demonstrated that most circRNAs did not function as miRNA sponges through bioinformatics and experimental verifications [122]. It is also unlikely for most circRNAs to function as templates for translation, as a limited number of circRNAs associate with polyribosomes [35]. The history of the role of long noncoding RNAs (lncRNAs) in biological processes, which have shown tremendous diversity, would be of some help for us to prospect the development of circRNA biology. In the same way, it would be more exciting to see new subsets of functional circRNAs and diverse functions and functional mechanisms that circRNAs exert in cancers. It is also logical to speculate that additional chemical modifications to circRNAs would hamper or enhance their capability in multiple cancers. For example, m^6A, the most abundant internal modification of RNAs in eukaryotes, could facilitate circNSUN2 export and promote colorectal liver metastasis [40]. However, much more needs to be known about the effects of m^6A modification on cellular circRNAs biology, as well as other modifications. What's more, aberrant expressions of circRNAs in cancers might result from genetic and/or epigenetic changes due to certain mutations of key regulators such as some spliceosome genes [123]. Recent findings also reported circRNAs from specific sites such as vesicles might function as signaling molecules that could be explored for clinical applications. More combinatory and integrated clinical studies, as well as novel drug delivery systems [124,125], are also required for the validation of targetable circRNAs in the diagnosis and treatment of specific cancers, since most of circRNA biomarkers in one cancer type might not apply to varying cancer models due to their cell type-specificity [126,127]. Despite our knowledge on the functional circRNAs in cancers is yet to be fueled, the growing number of evidence, together with the advent of novel bioinformatics analyses tools, are still generating promising future of new hypotheses and novel applications of circRNAs in diverse cancers.

Author contributions

G.S. and L.C. conceived the scope of the manuscript. L.C and G.S. wrote the manuscript. All authors discussed the manuscript and made comments on the manuscript.

Declaration of competing interest

We declare no competing financial interests.

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