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CircRNA: as a disease marker potential and research strategy

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Circular RNA (CircRNA) is an endogenous noncoding RNA with covalently closed cyclic structure. It is divided into exonic circRNA, intronic circRNA and exon-intron circRNA, based on their components. CircRNAs are well conserved in sequence and abundantly expressed in a tissue specific manner. They have a high stability due to resistance to exonuclease. Depends on their sequence, they perform many biological function including microRNA sponging activity, modulation of alternative splicing or transcription, interaction with RNA binding proteins, rolling translation and derivative of pseudogenes. They are involved in the development of a variety pathological condition including cardiovascular disease, diabetes, neurological diseases and cancer. Emerging evidences show that circRNA are likely to be potential targets for new clinical diagnostic markers or treatment of many diseases. In this review, we have described the potential relationship between circRNA and disease progression, methods and databases of cyclic RNA.

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Abstract: Circular RNA (CircRNA) is an endogenous noncoding RNA with covalently closed cyclic structure. It is divided into exonic circRNA, intronic circRNA and exon-intron circRNA, based on their components. CircRNAs are well conserved in sequence and abundantly expressed in a tissue specific manner. They have a high stability due to resistance to exonuclease. Depends on their sequence, they perform many biological function including microRNA sponging activity, modulation of alternative splicing or transcription, interaction with RNA binding proteins, rolling translation and derivative of pseudogenes. They are involved in the development of a variety pathological condition including cardiovascular disease, diabetes, neurological diseases and cancer. Emerging evidences show that circRNA are likely to be potential targets for new clinical diagnostic markers or treatment of many diseases. In this review, we have described the potential relationship between circRNA and disease progression, methods and databases of cyclic RNA.

Keywords: CircRNA; Diseases; Research strategy; Database

1. Introduction

Circular RNA (circRNA) was considered a class of endogenous non-coding RNAs(ncRNA) [1], but now it has been confirmed that circRNA can be translated as a functional polypeptides to function [2-5]. CircRNA was first discovered as genomes for certain RNA viruses in 1970s and 1980s. Unlike linear ncRNAs, CircRNA are formed with different combination of sequences and domains, and based on that they can be divided into three categories: Exonic circRNA (ecRNA)[6], Circular intronic (ciRNA)[7] and Exon—intron circRNA (ElciRNA)[8](Table 1)(Fig. 1). Like other ncRNAs, the sequence and structure of circRNA determines its biological functions. CircRNA are mainly found in the cytoplasm and they are highly stable compared to other ncRNAs [9] . They are abundantly expressed and evolutionarily conserved across the eukaryotic organisms [10,11]. CircRNA play very important role in many diseases including nervous system disorders, cardiovascular diseases, diabetes and cancer [12,13]. CircRNA have special features in biological system and their functions are tissue specific. CircRNAs govern gene expression through guiding a number of other molecules, such as splicing factors, RNA polymerase II[14], nuclear small ribose nucleoprotein (snRNP) [8,15] and miRNAs [16]. These interactions can promote or inhibit the transcription of the corresponding mRNA.

Survey Methodology

Analysis : Through extensive reading of the literature, the role of circRNA in diseases and research methods were analyzed, indicating the importance of circRNA and its research prospects: including the author's own previous research results combined with others' research, comprehensive analysis of the role of circRNA.

Subheadings

Disease marker potential; Research strategy

2. Biogenesis of CircRNA

CircRNAs do not have terminal structures such as 5' end cap and 3' end poly (A) tail, which are covalently closed to form a circular structure [14]. Jeck and his colleagues have proposed two different models of exon circularization. One is intron-pairing-driven circularization (Fig. 2a) and another model is lariat-driven circularization (Fig. 2b) [14]. The former is formed by the cyclization of ends of exons due to the complementary sequences of the introns. The matching between the reverse complementary sequences of introns leads to spatially closer and backward shear ring formation, which subsequently forms intronic circRNA by pairing the splice donor and acceptor sites. Typically, many lasso structures are formed by introns, but they are cleared by the branching enzyme degradation [17]; In lariat-driven circularization, heterogenous RNA (hnRNA) take parts in the process of RNA folding that brings exons closer to neighbouring exons and jump exons (Exon skipping), which results in generation of lasso intermediates and further splicing producing a mature exonic circRNA (ecRNA). Interestingly, all exons cannot form a circRNA, only the exons with reverse complementary Alu elements on both sides can pair and circularize, but it is not specifically required if there are other inverted repeats exist in the sequence [18]. DHX9 is a RNA helicase, which specifically binds to reverse Alu elements to guide the formation of cyclic RNA [19]. In addition, if the size of flanking introns is greater than that of exons, it favours easier cyclization [20]. Many proteins are involved in cricRNA biogenesis. The double-stranded RNA-binding domain containing immune factors NF90/NF110 are key molecules in circRNA biogenesis. NF90/NF110 promotes circRNA production in the nucleus by associating with intronic RNA pairs juxtaposing the circRNA-forming exon(s) [21]. HNRNPL promotes circular RNA formation via back splicing [22]. The RNA binding proteins such as MBL (muscleblind) [23], QKI (RNA-binding protein quaking I) [24] and FUS [25] also participate in the back-splicing process and cyclization of RNA. Interestingly, MBL promotes its own mRNA cyclization due to enrichment of putative MBL binding sites in flanking introns by which it connecting the flanking introns and sustaining cyclic structure and thus it promotes the exon cyclization. The monomeric QKI binds to both ends of intron flanking sites and they combined to form cyclic exons by bringing the two cyclic shear sites very close. FUS regulates circRNA biogenesis by binding the introns flanking the back-splicing junctions and this control can be reproduced with artificial constructs [25]. In contrast, the RNA editing enzyme ADARs (Adenosine deaminases acting on RNA) block circRNA formation by binds to complementary double-stranded area of flanking introns and abolishing the interaction of double stranded chains [26]. A recent study found that inhibition or slowing of pre-mRNA processing machineries such as spliceosomes leads to profound increase of circRNA production and this also enables the extend of read through s to downstream genes and production of circRNA [27].

3. Properties of CircRNA

CircRNA have several unique features and properties when compared with other linear RNAs as well as ncRNAs. A vast majority of them reside in the cytoplasm [29] and most of them are generated from exons, while few others come from introns or intron fragments. Several circRNA possesses microRNA response element (MRE), which enable them to interact with miRNAs and thereby they governs the target gene expressions [18,30]; Many circRNAs are

derived from pre-mRNAs and they regulate their own gene expression predominantly at post-transcription level [31] and only few of them regulate transcription [7] Generally, circRNA show tissue-specific and/or developmental-stage-specific expression pattern similar to that of corresponding linear mRNA targets, and their expression level is >10 times higher than of the linear mRNA [14] [32]. CircRNAs exist and detectable in many types of extracellular body fluids, such as saliva, blood, and urine [33] [14]. More than 400 circRNA have been found in human cell-free saliva (CFS) from healthy individuals [34]. CircRNAs show evolutionarily conserved sequence features across different species [35]. The covalently closed loop structures with lack of 5'-3' polarity and without polyadenylated tail fovours resistance to RNA exonuclease degradation [36]. CircRNAs play diverse biological roles due to the fact that average half-life of circRNA in most species is much longer than its linear counterpart [32,34].

4. Function of CircRNA

circRNAs have a variety of functions including miRNA sponging activity, modulation of alternative splicing or transcription, regulating the expression of parental genes, interaction with RBPs and alter their activity, rolling circle translation and generate pseudogenes.

4. 1 CricRNA as MiRNA Sponges

CircRNAs act as a competing endogenous RNAs (ceRNA) that contain shared miRNA response elements (MRE) by which they sequestering miRNAs and preventing their interactions with target mRNAs (Fig. 3a). The systematically validated circRNAs such as circRNA such as ciRS-7 (CDR1as) [18,32] and Sry circRNA(circSry) [18,37] are produced from the mRNAs of cerebellar degeneration associated protein 1 (CDR1) and dysregulated rat testis SRY respectively. During embryonic developmental process in zebrafish, the expression of CDRlas reduces the brain volume and its development is hampered. However, the exogenous delivery of miR-7 can bring the brain volume and development to normal, which illustrate that CDRlas blocks miR-7 by sponging functions [18,38]. New research finds that CDR1as sequence is overlapping the sequence of lncRNA LINC00632 [39]. Another abundant circRNA derived from Exon2 of the HIPK3 gene (circHIPK3) promotes cell proliferation by sponging miR-124. Interestingly, the silencing of circHIPK3 but not HIPK3 mRNA inhibits cell growth. The luciferase screening assay found that circHIPK3 possesses 18 potential binding sites to sponge 9 miRNAs. It can directly bind to miR-124 and inhibits its activity [40]. In generally, there are only few circRNAs containing enough miRNA-binding sites to function as a strong sponge and other circRNA are exceptional cases [43, 44]. Another study found that knockdown of circHIPK2 expression significantly inhibited astrocyte activation via the regulation of autophagy and endoplasmic reticulum (ER) stress through the targeting of MIR124-2HG and SIGMAR1 [41]. CircHECW2 plays a role in the epithelial-mesenchymal transition (EMT) pathway by competitively inhibiting miR-30D, releasing ATG5, and thereby promoting the Notch1 signaling pathway [42].

4. 2 CircRNA Modulates Alternative Splicing or Transcription

CircRNAs participate in the regulation of alternative splicing and transcription, and thereby they control gene expression (Fig. 3b). For example, circMbl is generated from the second exon of the splicing factor MBL, which competes with canonical premRNA splicing. circMbl and its

flanking introns have conserved MBL binding sites that can strongly bind with MBL. Interestingly, the alteration of MBL level significantly affects circMbl formation and this effect depends on MBL binding sites in the flanking intronic sequences [23]. Studies have shown that some circRNA are abundantly found in the nucleus and they regulate transcriptional activity by interaction with polymerase II and homeopathic reaction. For instance, EIciRNAs interact with small nucleonucleo proteins (snRNPs) to regulate the transcription of parental genes in a homeopathic manner [43]. Li et al. found that cir-ITCH interacts with miR-7, miR-17 and miR-214 and up-regulates the expression of ITCH [16]. During embryogenesis, sisR-4 promotes transcription of its host gene by activating an enhancer present in the intron where sisR-4 is encoded，which is essential for development [44]. HNRNPL directly regulates the alternative splicing of RNAs, including encoding the androgen receptor, the key lineage-specific prostate cancer oncogene [22].

4. 3 CircRNA Interacts with RBPs

Apart from miRNA regulation, circRNA can sequester RBPs and thus they control the intracellular localisation and transport of RBPs and its associated mRNAs [45,46](Fig. 3c). Some circRNA combine with RBP and ribonucleoprotein complex and prevent their activity, however, circRNA functions as stores of RBP and ribonucleoprotein complex. EcRNA acts as a scaffold by specifically binding with some protein molecules and providing interaction platform for RNA binding protein, RNA and DNA. For example, CDRlas combines with the miRNA effector protein, Ago2, and contributes to proteolysis function. Chen et al. found that there are cellular differential mechanisms in the recognition of internal and external circRNA. They found that the external circRNA induces activation of RIG-I-mediated cellular autoimmune effector pathways, while endogenous circRNA does not induce this pathway due to binding of RBPs [47].

4. 4 Rolling Circle Translation

In eukaryotic cells, the cyclic mRNA can be translated by typical translation machineries due to the fact that it contains an IRES (internal ribosome entry site) sequence and it can bind directly to the ribosome (Fig. 3d). In prokaryotic cells such as E. coli, the circRNA contains a well conserved ORF (infinite open reading frame) system, which enables the translation of circularised RNA [48]. In eukaryotic system, the some circRNAs have binding sites for ribosomal 40S subunits, thus it can initiate the translation. This was proved in both in vivo and in vitro studies. In E. coli system, the circRNA with an insertion of GFP (green fluorescent protein) in open reading frame can successfully translate GFP [49]. Interestingly, circRNA also drive protein translation by methylation of adenosine N6 (m6A) [2]. The protein translated by circRNA can act synergistically with the protein expression products of the parent gene and they can function together. For example, circ-FBXW7 can translate a new protein that inhibits glioma [3]. Circ-ZNF609 can directly translate proteins that participate in muscle formation [5]. In prokaryotic cells, the proteins are generated from circRNA by means of rolling circle amplification (RCA) analogous to polymerase reaction in the eukaryotic translation system, which reveals that there is no need for multiple binding of translational machinery to the RNA template. The circular amplification not only produces long and repetitive peptide sequences, but also increases the productivity of the linear counterpart [50].

193 *4. 5 Generate Pseudogenes*

194 Studies have shown that stable circulatory molecules can be reversed transcribed and
195 integrated into the genome to form a circRNA-derived pseudogenes [51] (Fig. 3e). The
196 bioinformatic analysis of mouse genome using computational pipeline (CIRCpseudo) found that
197 at least 33 pseudogenes are possibly derived from the same circular RNA at the RFWD2 (ring
198 finger and WD repeat domain 2) locus (circRFWD2) and nine of those pseudogenes are from
199 exons (exons 2 to 4 or 5) of circRFWD2. It is well documented that pseudogenes play an
200 important role in the cell differentiation as well as in cancer progression [52].

201 **5. CircRNA in disease development and progression**

202 The best known circRNA such as ciRS-7 and CDR1as, the inhibitors of miR-7 microRNA, are
203 critical ncRNA known to be involved in various diseases including, cancer, neurodegenerative
204 diseases, diabetes, and atherosclerosis. Thus, the complex association of circRNA with critical
205 microRNAs and other gene families, circRNA might have important role in the development and
206 progression of various diseases (Table 2).

207 **6 Methods of CircRNA detection and characterisation**

208 *6. 1 Preliminary Purification and Identification*

209 6. 1. 1 Molecular biology method

210 The loop structure of CircRNA has high stability compared to Linear RNA and it is resistance
211 to enzyme digestion. Therefore, enzymatic digestion method can be used for the preliminary
212 purification and identification of CircRNA [46].
213 As a first step, the processing of extracted RNA with exonucleases such as RNase R,
214 nicotinic acid phosphatase and 5 'end exonuclease can destroy most linear RNA, but circRNA
215 remain intact due to no open ends in circRNAs for these enzyme reactions. The circRNA specific
216 divergent primer can be used to amplify abundant circRNA in which linear RNAs do not amplify
217 [46,94]. Second, the migration velocity of circRNA is slower than the long linear RNA due to lack
218 of polarity at the end. In particularly, their migration is much slower than RNA from
219 homologous gene transcription in the weak crosslinked gel. This difference helps to detect
220 them easily through Northern blot analysis [95]. Third, the fluorescence in situ hybridization
221 technique can locate circRNA in subcellular level [7,8]. As the circRNA do not have poly (A)
222 structure, the traditional oligo dT enrichment method, using ribo - zero kit to remove rRNA, is
223 not to be effective. The removal of linear RNA using RNase R is a most effective for the
224 enrichment of circRNA and library building [46,96].

225 6. 1. 2 High-throughput sequencing

226 The traditional RNA-seq technique does not distinguish the circRNA from linear RNAs. As a
227 result, the researchers have made some effective improvements in order to detect and validate
228 circRNA. First, as the intergenic exon rearrangement has different forms, building the divergent
229 primers with boundary combination can form circRNA candidate sequence and then it can be
230 compared with the sequencing data [97]. Second, the bioinformatic analysis of whole genome

sequence and assessement of sequence data through different sequence alignment algorithm [14];.Third, the designing of templates with multiple sequence splice joint, which can directly detect circRNA from the cDNA sequence [98]. Currently, many algorithms are available for the prediction and studying circRNA that includes Acfs [99], FUCHS [100], CIRI2 [101], etc. Acfs allows accurate and fast identification of circRNA. It also helps to determine the abundance of circRNA from single- and paired-ended RNA-Seq data. It is well suitable for a wide spectrum of applications including characterizing the landscape of circRNA from a variety of organisms. FUCHS system is based on long sequencing reads (> 150bp / Reads), which achieves the detection of a circular RNA within the variable shear and other information for more accurate interpretation. CIRI2 uses the maximum likelihood estimate based on multiple seed matches to identify the reverse splice junctions and it filters out false positives and mapping errors derived from the repetitive sequence. CIRI2 has a significant balance of sensitivity, reliability, duration and RAM usage[46, 96].

6. 1. 3 Gene chip

The human genome array, 133plus2.0 tool, can detect mRNA, but it does not able to detect the ring RNA because the probe is designed for linear RNA. So it cannot effectively distinguish cyclic RNA and linear RNA, when normal probe is used. However, if the probe is designed based on the reverse splice site of the circRNA, this array tool specifically detect circRNA, as there is no reverse splice site sequence on the linear RNA. In this way, it can effectively distinguish circular RNA and mRNA.

6. 1. 4 Primer Design

The field of circRNA research gaining more attention recently due to the fact that they contribute to many physiological as well as pathological processes. Unlike conventional PCR primers, the designing of the circRNA primers should consider the following criteria: For the detection of exonic circRNA, the primers for cross-cut site (backsplice) should be designed. In the case of intron cyclized cyclRNAs, the primers targeting cross-cleavable sites should be used. The primers can also be designed around intron regions. The length of the amplified product should not be more than 100 bp. The squence position transformation is also important. The difference in selection of primers for linear RNA and circRNA is given in Fig. 4.

The actual amplification effect after primer design needs to be experimentally determined. If the quantification of circRNA is carried out by qPCR, the length of amplification should be settled according to the experimental requirements of qPCR. Thus, qPCR remains the most widely used technique to assess the expression level of circRNA.

6. 2 The Database in CircRNA Research

In recent years, the number of cirRNA research tools is rapidly increasing with different aspects and improved functional analysis. Here, we present currently available online databases for the detection and characterization of circRNA, which contain GenBank annotations or circRNA from published articles. Each database analyses cricRNA with different characteristics features feeded for the detection and they provide much information about circRNA. There are several free online databases available for the circRNA research (Table 3).

8. Conclusion and Future Directions

CircRNAs have the characteristics of conserved sequence, tissue specificity, more stability and high abundance of expression, which enables them to be potential markers for disease screening and treatment. The rapid development of high throughput sequencing techniques and bioinformatic analyses suggest that it is likely to become a new efficient target in the clinical settings for the detection and treatment of diseases such as diabetes, cancer, cardiovascular disease and neurological diseases. It is notable that circRNAs could function as miRNA sponges and regulating multiple signaling pathways in the cardiovascular diseases, different types of cancer, neurodegenerative diseases and diabetes. However, further researches are needed to reveal the complete biological functions of of circRNA in terms of both physiological and pathological processes so that it can be applied to clinical use in future.

Despite the rapid advancement has been in the detection and characterization of circRNA, the knowledge about the functions of circular RNA is still at an early stage, which is one of the major drawbacks for the potential use of circRNA for therapeutic or diagnostic purposes. The new generation methods such as chip technology can be used to screen the possible disease-related circRNA in the cell or experimental animal models. This will increase our knowledge about the role of circRNA in the occurrence and development of pathological disorders. In addition to deepening the circRNA research in functional aspects, the someother questions should also to be refined. For example, how the dynamics of the circRNA formation is triggered and controlled? What is the link between circRNA formation process and the corresponding linear RNA generation? What is the relationship between different circRNA products from the same gene? How is the circRNA generation precisely regulated? The identification and characterisation of specific circRNA interacting molecules is important to answer most of these fundamendal questions. In addition, the naming of circRNA has not yet been unified and the mechanisms of circRNA in many diseases are not clear. By solving all these questions, circRNA could be a promising diagnostic tool for the detection and efficient therapeutic targets for treatment of various pathological disorders.

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310 **References**

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Table 1(on next page)

The characteristics of different types of CircRNA

ecRNA : Exon circRNA ; ciRNA : Intron circRNA ; ElciRNA : Exon-intron circRNA

Table 1 The characteristics of different types of CircRNA

Name	Type	Location	Joint Site	Sequence Feature	Function
ecRNA[6]	exon	cytoplasm	3'-5' phosphodiester bond	Formed by cyclization of exons containing the reverse complementary sequence of introns and selective cyclization.	Functioning as MiRNA Sponges; Interact with RNA Binding Proteins (RBPs); Participates in translation.
ciRNA[7]	intron	nucleus	2'-5' phosphodiester bond	5 'splice site enriched with 7 GU motif and 3' branch site contains 11 C motif.	Regulation of gene transcription.
ElciRNA[8]	exon - intron	nucleus	3'-5' phosphodiester bond	Formed by cyclization of exons containing the reverse complementary sequence of introns and selective cyclization.	Regulation of gene transcription.

ecRNA: Exon circRNA; ciRNA: Intron circRNA; ElciRNA: Exon-intron circRNA

Table 2(on next page)

CircRNA in disease development and progression

HRCR: heart-related circRNA ; Canril: circular antisense non-coding RNA at the INK4 locus ; MFACR: mitochondrial fission and apoptosis-related circRNA;

1 Table 2 CircRNA in disease development and progression

Diseases		CircRNA	Functions
Cardiovascular Diseases	Pathological hypertrophy and heart failure (HF)	HRCR	Mir-223 is a positive modulator of hypertrophy in cardiomyocytes, which can induce cardiac hypertrophy and heart failure [49,53], HRCR acts as an endogenous mir-223 sponge to inhibit the hypertrophic response [54].
	Atherosclerosis	CANRIL	CANRIL prevents rRNA pre-binding and exonuclease-mediated rRNA maturation by binding to the C-terminal lysine-rich domain of PES1, this pathway can inhibit atherosclerosis by eliminating hyperproliferative cell types in atherosclerotic plaques [55].
	Cardiac senescence	Circ-Foxo3	Circ-Foxo3 is generated from Foxo3. Its expression is highly correlate with markers of cellular senescence [56] and circ-Foxo3 represses cell cycle progression and cell proliferation [57], it interacts with several transcription factors (E2F1, FAK, and HIF1a) and anti-senescent protein such as ID-1 and preventing their nucleus entry, which repress their anti-senescence roles, circ-Foxp3 also positively correlates with cellular senescence [56].
	Myocardial Infarction(MI)	Cdr1as	Cdr1as promotes apoptosis and MI injury by blocking the activity of mir-7a and increasing the expression of mir-7a targets such as PARP and SP1[58-60].
		MFACR	MFACR dependent inhibition of mir-652-3p increases MTP18 and mitochondrial fission, which results in reduction of cardiomyocyte apoptosis

			and extension of MI injury [61].
Neurodegenerative Diseases		CDR1as	In lead-induced neuronal apoptosis, circRar1 can directly inhibit miR-671, which leads to the suppression of Akt2 and increased expression of Caspase-8 and other apoptosis-related proteins [62].
Osteoarthritis (OA)		Hsa_circ_0005105	It promotes extracellular matrix (ECM) degradation by regulating the expression of mir-26a target NAMPT [63].
Major depressive disorders (MDD).		Hsa_circRNA_103636	It is easily detectable in blood samples and its expression pattern altered in MDD [64].
Silicosis		CircHECTD1	It mediates silica-induced macrophage activation via HECTD1/ZC3H12A-dependent ubiquitination [65].
Diabetes		Cdrlas	The impairment of Islet β-cell function leads to a absolute or relative insulin deficiency (insulin resistance), which causes increase in blood sugar level and diabetes [66,67], mir-7 negatively regulates the proliferation of islet β cells and targets multiple components of mammalian target of ripamycin (mtor) signaling pathway, which are involved in pancreatic β cell proliferation, these finding reveal that CDR1as/mir-7 could be a potential therapeutic target for treating and managing diabetes [68].
Cancer	Gastric Cancer (GC)	Hsa-circ-002059	The increased expression of Hsa-circ-002059 is significantly associated with the tumor stage of GC [69].
		Circrna_100269	Circrna_100269 can suppress gastric tumor cell growth by targeting mir-630. However, its expression is

			downregulated during GC [70].
		Hsa_circ_0003159	The expression of hsa_circ_0003159 is negatively associated with tumor-node-metastasis stage [71].
		Hsa_circ_0000190	The increased expression of hsa_circ_0001895 is significantly correlated with tissue carcino-embryonic antigen expression [72].
		CircPVT1	It promotes cell proliferation by acting as a sponge for members of miR-125 family and often upregulated in GC tissues [73].
	Colorectal Cancer (CRC)	Hsa_circ_001988	The expression of hsa_circ_001988 is downregulated and it is associated with differentiation and perineural invasion in CRC [74].
		Circ_001569	Circ_001569 directly inhibits the regulatory activity of mir-145, and thereby it up-regulates the expression of its protein targets such as E2F5, BAG4 and FMNL2 , which are involved in tumor proliferation and invasion in CRC [75].
		Hsa_circ_0000069	It upregulates cell proliferation, migration, and invasion [76].
		CircCCDC66	Circccdc66 regulates a subset of oncogenes, which control multiple pathological processes, including cell proliferation, migration, invasion, and anchorage-independent growth in CRC [77].
	Esophageal Squamous Cell	Has_circ_0067934	Has_circ_0067934 is upregulated and it accelerates malignant cell proliferation [78].

	Carcinoma (ESCC)	Cir-ITCH	Cir-ITCH can inhibit ESCC proliferation by suppressing Wnt/-catenin pathway through sponging activity on miRNAs such as mich-7, mir-17 and mir-214 and thereby it enhances expression of ITCH [16].
	Hepatocellular Carcinoma (HCC)	CircZKSCAN1	CircZKSCAN1 inhibits HCC cell growth, migration, and invasion by blocking several signaling pathways [78].
		Cdrlas	Mir-7 is a tumor suppressing ncRNA, which attenuates HCC proliferation and it decreases the risk of microvascular invasion (MVI) by suppressing its target gene PIK3CD and p70s6k expression. However, miR-7 activity is counteracted by the overexpression of cdrlas, which adsorbs miR-7 [79].
		Hsa_circ_0005075	Hsa_circ_0005075 participates in cell adhesion during HCC development [80].
		Hsa_circ_0004018; hsa_circ_0001649; circ-ITCH	They are significantly downregulated in HCC [81-83].
		Hsa_circ_0085154	It is downregulated by androgen receptor (AR) dependent activation of ADAR1 expression in both HCC cells and malignant tissue [84].
	Cervical Cancer	Cdrlas	FAK promotes the proliferation, invasion and migration of cervical cancer cells and that exacerbates the progression of the disease. CDRlas promotes FAK expression by inhibiting miR-7, which targets FAK

			[85].
		circRNA-000284	It promotes cell proliferation and invasion in cervical cancer [86].
	Breast Cancer	Circ-Amotl1	Many circRNA are differentially expressed in breast cancer [87]. Hippo signaling promotes breast cancer progression by upregulating the expression of AMOTL1 and favouring metastasis [88]. Circ-Amotl1 interacts with c-myc and translocates to nucleus [89].
	Human oral squamous cell carcinomas (OSCC).	Circrna_100290	It acts as a competing endogenous RNA to regulate CDK6 expression through sponging up miR-29b family members [90].
	Lung adenocarcinoma (LAC)	Hsa_circ_0013958	It promotes cell proliferation and invasion. It inhibits cell apoptosis [91].
	Bladder carcinoma	CircTCF25	It downregulates mir-103a-3p and mir-107, and increases the expression of CDK6. It promotes cell proliferation and migration [92].
		CircPTK2	It promotes proliferation and migration of bladder cancer cells [93].

HRCR: heart-related circRNA; Canril: circular antisense non-coding RNA at the INK4 locus; MFACR: mitochondrial fission and apoptosis-related circRNA;

Table 3(on next page)

The Database for CircRNA Research

Every database present has its own sphere of competence, only the perfect combination of various database can provide information accurate.

1 **Table 3** The Database for CircRNA Research

Tool Name	The latest version	URL	Remarks
circlncRNAnet [102]	May 2017	http://app.cgu.edu.tw/circlnc/	It aims to broaden the understanding of ncRNA candidates by testing in silico several hypotheses of ncRNA-based functions, on the basis of large-scale RNA-seq data.
starBase v2. 0[103]	December 2013	http://starbase. sysu. edu. cn/	Including microRNA, mRNA, lncRNA and other RNA information. It is a most useful tool for detecting miRNA-circRNA interaction. If there is need for retrieve all circRNAs in the genome, circRNABase is useful.
circBase[104]	December 2015	http://www. circbase. org/	Thousands of circular RNAs (circRNA) are annotated from eukaryotic cells.
circ2Traits[105]	December 2013	http://gyanxet-beta. com/circdb	Provides more information about the genomic positions of circRNA and cirRNA-associated diseases.
nc2Cancer[106]		http://www. Bioinfo. Tsinghua. Edu. cn/nc2Cance	
CircNet[107]	December 2015	http://circnet. mbc. nctu. edu. tw/	A database of circular RNAs derived from transcriptome sequencing data and this tool has most CircRNA annotation, in particularly from human.
deepBase v2. 0[108]	November 2015	http://biocenter. sysu. edu. cn/deepBase/	This database is a platform for annotation and discovery of small (microRNAs, siRNAs, piRNAs.) and long ncRNAs from next generation sequencing data.
CircInteractome[109]	December 2015	http://circinteractome. nia. nih. gov/	This database can be used only to match the circRNA with relevant RNA binding proteins.
TSCD[110]	August 2016	http://gb. whu. edu. cn/TSCD/	It is useful for characterizing tissue-specific circRNA in human and mouse genomes.
CIRCpedia[111]	January 2015	http://www. picb. ac. cn/rnomics/circpedia/	This database contains reverse splicing and variable splicing sites of circRNA from 39 individuals and mouse samples.
circRNADb[11		http://reprod. njmu. edu.	It contains a record for more

2]	cn/circrnadb	than 30,000 exons with cyclic RNA nature in human genome

Every database present has its own sphere of competence, only the perfect combination of various database can provide information accurate.

Figure 1

Characteristics of Different Types of CircRNA

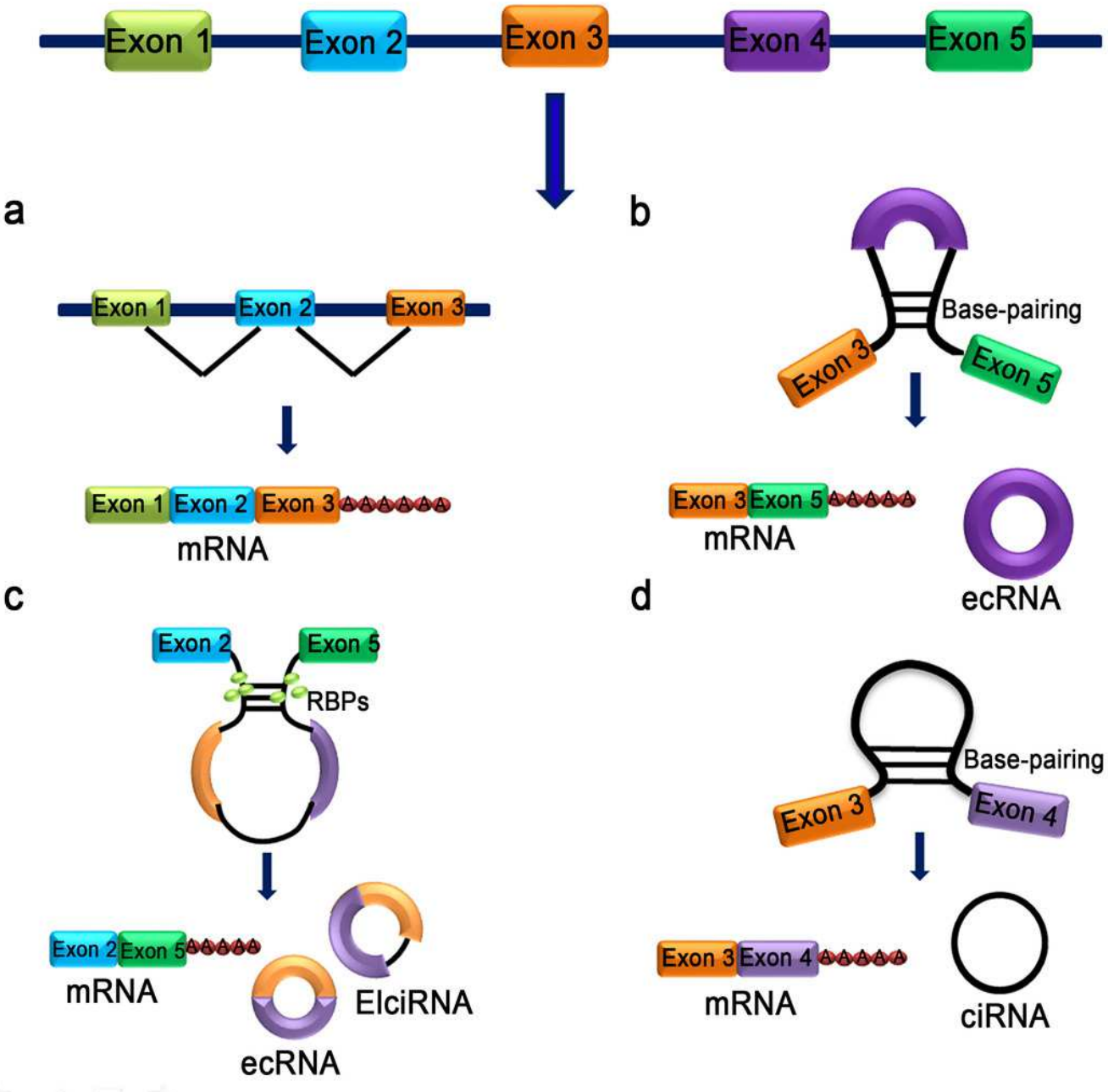

Figure 2

Two Different Models of Exon Circularization of CircRNA

'(a) intron-pairing-driven circularization : d uring the formation of circRNA, an intron reverse complementary motif comprising GU-rich and C-rich elements is the key component to facilitate cyclization. b) lariat-driven circularization : t he formation of circRNA is facilitated by the lariat structure. The complementary ALU flanking elements which is repeated in the intron region compete for classical linear RNA splicing and the circularization is accelerated by reverse complementarity [28] . '

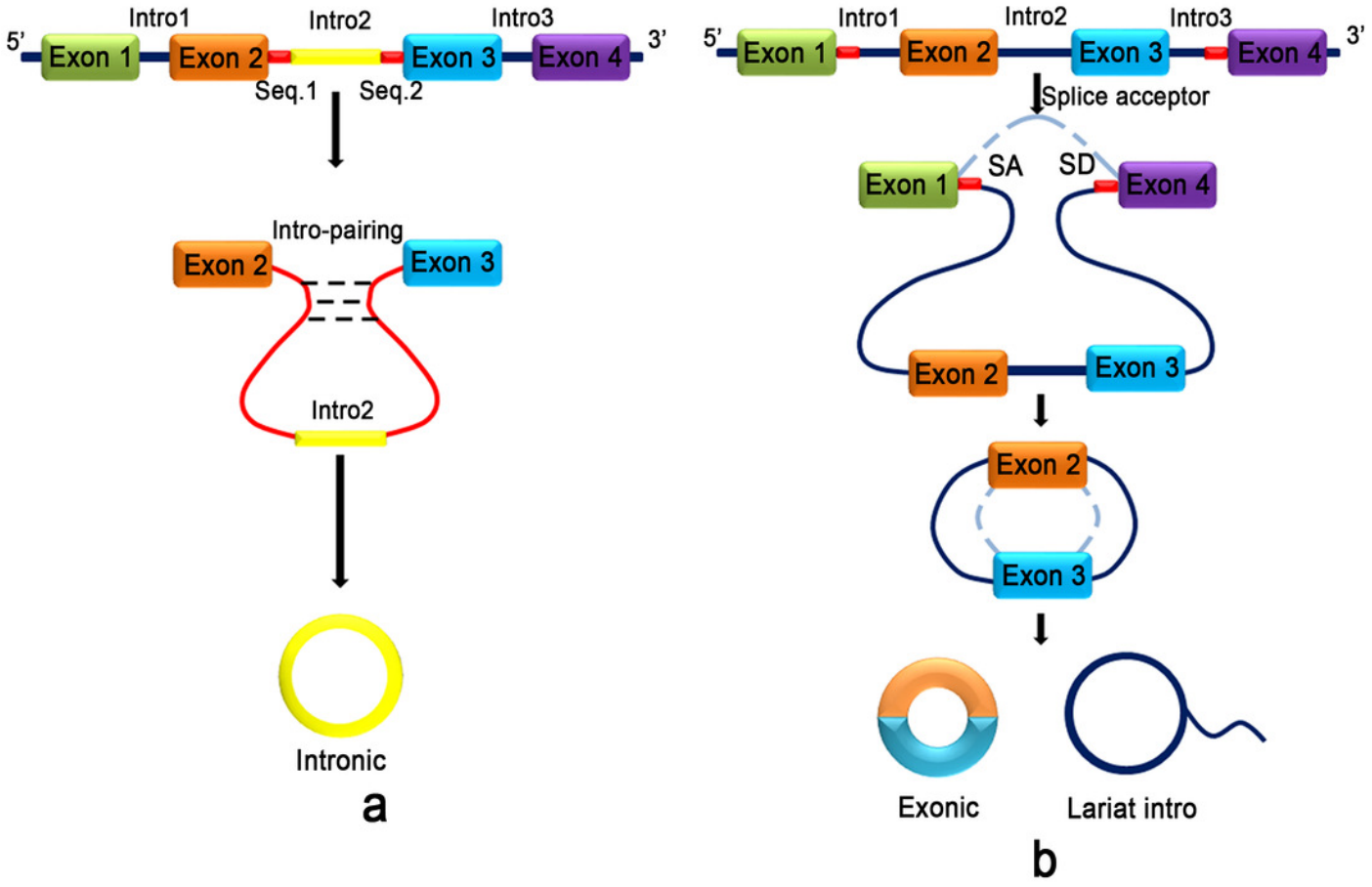

Figure 3

The five main functions of the circRNA

'(a) miRNA sponging activity: circRNA binds with miRNA and affecting the miRNA dependent target gene suppression.(b) Regulating selective splicing or transcription: Stable circRNA and ElciRNAs are located in the nucleus, where they bind to RNA polymerase and promoting transcription; circRNA competes with pre-mRNA splicing to reduce the level of linear mRNA and excludes specificity from pre-mRNA by changing the composition of processed mRNA. (c) Interaction with RBPs: circRNA binds with RBPs and ribonucleoprotein complexes and interfere with their functions . As a single circRNA can bind with multiple units of RBPs, they serve as stores of RBPs. (d)Rolling Circle Translation: Some circRNA can be translated into proteins by means of a roll loop amplification mechanism [28]. (e) Generation of Pseudogenes: Some circRNA are reverse transcribed into cDNA and integrated into the genome, however, the mechanism of integration is not yet clear. '

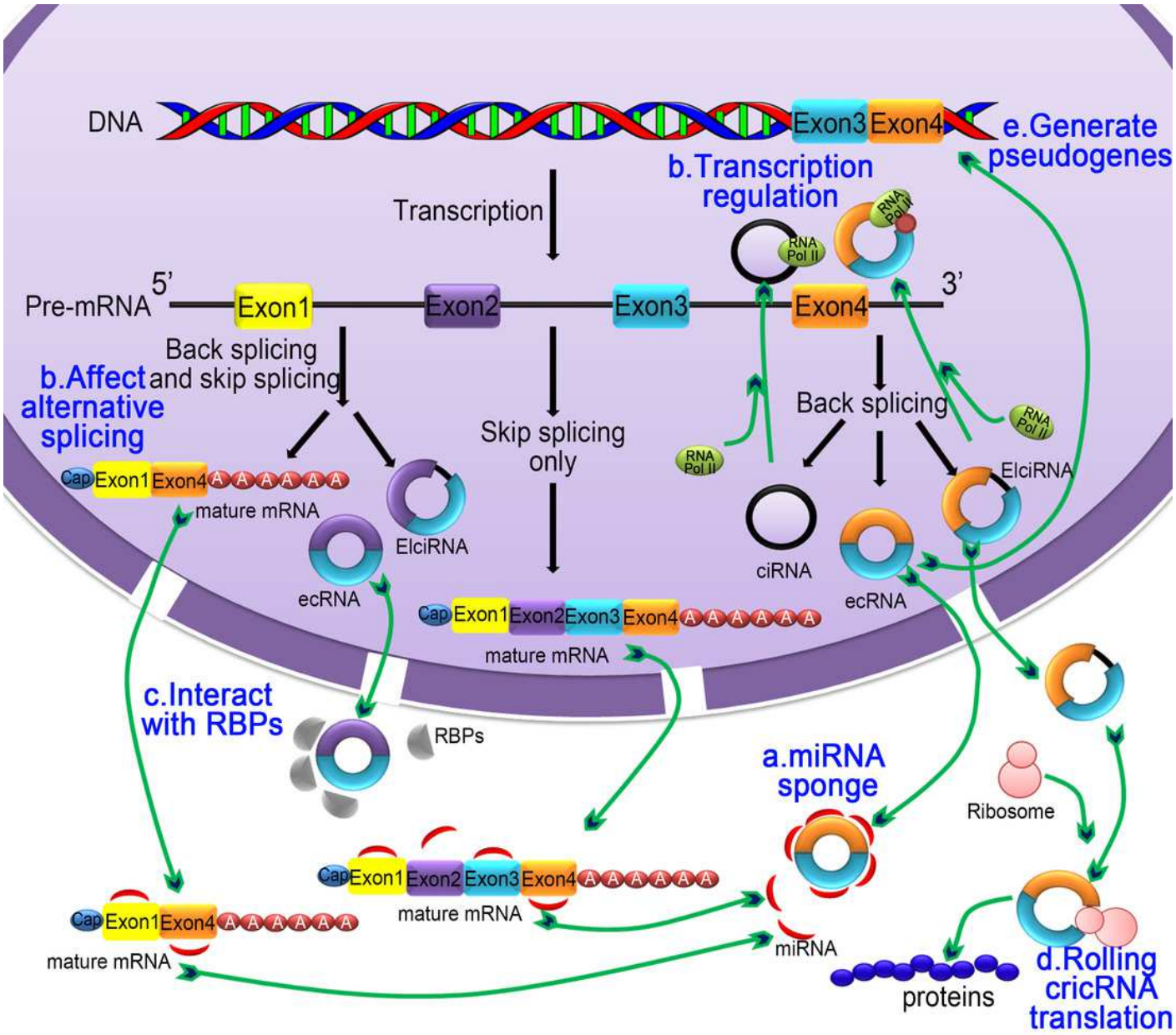

Figure 4

The Difference between Linear RNA and CircRNA primerdesign

'(a) FW is a forward primer with B chain as template. The base sequence of synthesis is the original sequence of A; RV is a reverse primer with A chain as template, and the base sequence of synthesis is the original sequence of B, The sequence between FW and RV is high. (b) Need to reverse the original primers: The synthetic primers are FW' and RV', where FW' is the reverse complementary sequence of the RV primer, RV' is the reverse complementary sequence of FW primer. '