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Review

# Circular RNAs: Promising Biomarkers for Human Diseases

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ABSTRACT

Circular RNA (circRNA) is a group of endogenous noncoding RNA characterized by a covalently closed cyclic structure lacking poly-adenylated tails. Recent studies have suggested that circRNAs play a crucial role in regulating gene expression by acting as a microRNA sponge, RNA binding protein sponge and translational regulator. CircRNAs have become a research hotspot because of their close association with the development of diseases. Some circRNAs are reportedly expressed in a tissue- and development stage-specific manner. Furthermore, due to other features of circRNAs including stability, conservation and high abundance in body fluids, circRNAs are believed to be potential biomarkers for various diseases. In the present review, we provide the current understanding of biogenesis and gene regulatory mechanisms of circRNAs, summarize the recent studies on circRNAs as potential diagnostic and prognostic biomarkers, and highlight the major advantages and limitations of circRNAs as novel biomarkers based on existing knowledge.


**Contents**



## 1. Introduction

The recent advances in molecular biology techniques enable researchers to explore the complex mediatory network of coding and noncoding transcriptome. Circular RNAs (circRNAs) are a novel class

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of endogenous noncoding RNAs and a field of much research interest and activity. Unlike linear RNAs, such as mRNAs, microRNAs (miRNAs) and long noncoding RNAs (lncRNAs), with a 5′ cap and 3′ tail structure; circRNAs are characterized by a covalently closed loop structure formed by back-splicing event. As early as 1970s, circRNA molecules were first discovered in RNA viruses by scientists with electron microscope. However, back then the circularized RNAs were thought to be splicing artefacts; and were continuously considered as "junk" RNAs for about two decades until the recent developments in transcriptome sequencing and bioinformatics analysis. Currently, circRNAs have emerged as the most interesting molecules because of their high abundance, stability, and conservation in mammalian cells [1]. CircRNAs have been reported to orchestrate gene expression by acting as miRNAs sponges, interacting with RNA binding proteins (RBPs) and modulating transcription. Importantly, tissue-specific regulation of circRNAs expression has been found associated with initiation and progression of numerous diseases, including various kinds of cancers, cardiovascular diseases, and neurological diseases [2–4]. Here we summarize the present understanding of the formation and function of circRNAs in disease development and discuss the feasibility of circRNAs to serve as biomarkers for different human diseases.

## 2. Biogenesis and Classification of Circular RNAs

CircRNAs are mainly synthesized by the transcription of protein-coding genes with RNA polymerase II (Pol II); but unlike linear RNAs, they are not produced by canonical mode of RNA splicing [5]. CircRNA molecules are circularized by joining the 3′ and 5′ ends together with unique back-splicing [6]. CircRNAs are commonly named according to their parental genes or specific functions, for example cerebellar degeneration-related protein 1 antisense RNA (CDR1as) is also known as ciRS-7 (circRNA sponge for miR-7) [7]. In this way, the same circRNA may be described with distict names by different researches. Recently, along with the ongoing reseach of circRNAs, several circRNA databases have been constructed to enable orgnization of discovered and idntified circRNAs. A serial number is given to every detected back-spliced juntoin site. Databases like circBase (http://www.circbase.org/) and CircNet (http://circnet. mbc.nctu.edu.tw/) provides tissue-specific circRNA expression profiles as well as circRNA-miRNA-gene regulatory networks [8,9]. Circ2Traits (http://gyanxet-beta.com/circdb/) also allows user to search circRNAs by mutiple diseases [10].

Based on the containing components of exons and introns from the parental genes, circRNAs can be divided into three categories: exonic circRNAs (ecircRNA) that only contain back-spliced exons; circular intronic RNAs (ciRNA) that come from introns; and exon-intron circRNAs (EIciRNAs) which is circularized with both exons and introns [11]. Two different models of exon cyclization have been proposed by Jeck and colleagues, which is termed "lariat-driven circularization" and "intron-pairing driven circularization" (Fig. 1) [12]. The former model is associated with "exon skipping", which leads to a covalent splice from the 3′ end of splice donor to 5′ end of splice acceptor, resulting in an exon-containing lariat structure. The lariat is then joined by spliceosome and form an exonic circle after the introns being removed (Fig. 1a). The latter one is based on pairing of complementary motifs in the transcripts. EcircRNA and EIciRNAs are formed respectively by removing or retaining the introns. Complementary flanking Alu elements are suggested important for circRNA biogenesis, whereas other inversed repeat sequences are also sufficient to drive circRNA formation (Fig. 1b) [13]. Accumulating evidence has verified the model of intron pairing driven circularization suggesting it might occur more frequently than lariat-driven circularization [14]. Thereafter, a new type of circRNA derived from intron was discovered in human cells and a novel model of ciRNA formation due to failure in debranching was proposed (Fig. 1c) [15]. Additionally, recent studies also suggested that circRNAs can be generated by bridging of RNA molecules with RNA binding proteins (RBPs). RBPs such as Muscleblind (MBL) protein and Quaking (QKI) protein can bind to flanking introns and mediate the circularization (Fig. 1d) [1,5].

## 3. Putative Mechanisms of Gene Regulation by Circular RNAs

### 3.1. Competing Endogenous RNA or miRNA Sponges

Competitive endogenous RNA hypothesis is currently the most intensively studied and well accepted mechanism on regulatory function of circRNAs on gene expression. CircRNAs contain plenty of miRNA response elements (MREs) that allow them to competitively bind to miRNAs, leading to decreasing of the functional miRNA molecules and subsequent upregulation of target miRNAs [6,16]. This phenomenon is also described as miRNA sponge since circRNAs can "absorb" miRNAs like a sponge (Fig. 2a). CDR1as is the most representative miRNA sponge circRNA. It has been reported contain more than 70 conserved binding sites for miR-7, and therefore pronouncedly reduced miR-7 level when elevated. Similarly, circ-SRY (sex-determining region Y), which is responsible for mammalian sex determination and specifically expressed in testis, has 16 binding sites for miR-138 [7]. Additionally, circ-HIPK3, HRCR and many other circRNAs have all been documented with miRNA sponge function [17–19]. Although sponge effect is a classical model of circRNA-mediated gene regulation, some recent studies have revealed that only few circRNAs exhibit properties of miRNA sponges and physiological ceRNA expression changes do not have impact on highly expressed miRNAs [20,21]. The interaction between circRNAs and miRNA are regarded also related to storage, sorting and localization of miRNA other than simple inhibition [22].

### 3.2. Interaction with RNA Binding Proteins and mRNAs

In addition to the role of circRNAs as miRNA sponge, circRNAs can also act as sponges for RBPs (Fig. 2b). For example, strong direct interaction between circMbl and MBL protein enable circMbl to function in gene regulation by competing with linear splicing [5]. CircPABPN1 suppresses PABPN1 translation by competitively binding to HuR preventing interaction between HuR with PABPN1 miRNA [23]. Circ-Foxo3 can repress cell cycle progression by binding to G1 to S phase transition-related CDK2 and p21 [24]. Besides, circRNAs are able to interact with mRNAs as well. CircRNAs that contain translation start site can act as mRNA traps and regulate protein translation of mRNA [14]. Moreover, several circRNAs have been reported capable of modulating the stability of mRNAs. CDR1as is suggested to form a duplex structure with CDR1 mRNA which in turn stabilizes it [22]. LPS-inducible mouse circRNA circRasGEF1B has been found facilitate the stabilization of mature ICAM-1 mRNAs in macrophages [25].

### 3.3. Regulation of Parental Gene Transcription

CircRNAs have also been reported to regulate transcription of parental gene through distinctive mechanisms (Fig. 2c). CiRNAs that are enriched in nuclei can interact with Pol II machinery and modulate host transcription activity in a cis-acting manner [15]. Nuclear EIciRNAs containing intronic sequence from their parental gene, such as circEIF3J and circPAIP2, can interact with the U1 small nuclear ribonucleoproteins (snRNPs) which then bind to Pol II on the promoter of their parental genes and thus enhance gene expression [11]. Also, circ-ITCH and the 3′-untranslated region (UTR) of ITCH gene share some identical miRNA binding sites with miR-1, miR-17 and miR-214, and thereby circ-ITCH regulates the expression level of ITCH by indirectly by interacting with its target miRNA [18].

### 3.4. Protein Translation

Recent studies have demonstrated the potential of circRNAs in proteins translation (Fig. 2d). The first natural circRNA found able to encode

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