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Mini-review

Circular RNA: A new star of noncoding RNAs

Shibin Qu [1], Xisheng Yang [1], Xiaolei Li [1], Jianlin Wang, Yuan Gao, Runze Shang, Wei Sun, Kefeng Dou, Haimin Li *

Department of Hepatobiliary Surgery, Xijing Hospital, The Fourth Military Medical University, Xi'an, 710032, China

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ABSTRACT

Circular RNAs (circRNAs) are a novel type of RNA that, unlike linear RNAs, form a covalently closed continuous loop and are highly represented in the eukaryotic transcriptome. Recent studies have discovered thousands of endogenous circRNAs in mammalian cells. CircRNAs are largely generated from exonic or intronic sequences, and reverse complementary sequences or RNA-binding proteins (RBPs) are necessary for circRNA biogenesis. The majority of circRNAs are conserved across species, are stable and resistant to RNase R, and often exhibit tissue/developmental-stage-specific expression. Recent research has revealed that circRNAs can function as microRNA (miRNA) sponges, regulators of splicing and transcription, and modifiers of parental gene expression. Emerging evidence indicates that circRNAs might play important roles in atherosclerotic vascular disease risk, neurological disorders, prion diseases and cancer; exhibit aberrant expression in colorectal cancer (CRC) and pancreatic ductal adenocarcinoma (PDAC); and serve as diagnostic or predictive biomarkers of some diseases. Similar to miRNAs and long noncoding RNAs (lncRNAs), circRNAs are becoming a new research hotspot in the field of RNA and could be widely involved in the processes of life. Herein, we review the formation and properties of circRNAs, their functions, and their potential significance in disease.

Introduction

Circular RNAs (circRNAs) were recently discovered as a special novel type of endogenous noncoding RNA and represent a recent research hotspot in the field of RNA. Unlike linear RNAs that are terminated with 5′ caps and 3′ tails, circRNAs form covalently closed loop structures with neither 5′–3′ polarities nor polyadenylated tails [1].

CircRNA was first found in RNA viruses as early as the 1970s [2]. Unfortunately, only a handful of such circRNAs were serendipitously discovered over the past 30 years [3–9]. Such molecules were typically considered to be molecular flukes or products of aberrant RNA splicing due to their low levels of expression. However, with the development of RNA deep sequencing technology and bioinformatics, recent work has revealed that large numbers of circRNAs are endogenous, abundant, conserved and stable in mammalian cells [10–16]. Furthermore, several researchers have confirmed that reversed complementary sequences including inverted repeated Alu pairs (IRAlus) and exon skipping are essential to circRNA formation [17–25]. Moreover, RNA-binding proteins (RBPs) also regulate circRNA formation [23,26].

Specifically, subsequent reports revealed that circRNAs could function as microRNA (miRNA) sponges, regulate alternative splicing, and modulate the expression of parental genes [13,14,16,23,27]. More importantly, it is becoming evident that circRNAs may be involved in atherosclerotic vascular disease risk, neurological disorders, prion diseases and cancer [28–30]; are aberrantly expressed in colorectal cancer (CRC) [31] and pancreatic ductal adenocarcinoma (PDAC) (S.B.Q., unpublished observations). CircRNAs were described as potential disease biomarkers in human saliva and as biomarkers for aging and gastric cancer (GC) [32–34]. Taken together, these findings indicate that circRNAs have great potential to perform special regulating roles in biological development and disease initiation and progression, become new clinical diagnostic and prognostic markers, and provide new insights into the treatment of diseases.

In this review, we briefly delineate the diversity of circRNAs and discuss the highlights of the biogenesis of circRNAs, their characteristics, their potential functions and their relationships with the disease.

Diversity of circRNAs

CircRNAs are expressed at low levels and were originally thought to be by-products of spliceosome-mediated splicing errors [35] or intermediates that escaped from intron lariat debranching [36,37]. Thus, circRNAs received little attention and were thought to be

* Corresponding author. Tel.: +86 29 84771098; fax: +86 29 84771098.
E-mail address: lihaim@fmmu.edu.cn (H. Li).
[1] These authors contributed equally to this work.

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Table 1
Overview of human circRNAs identified recently.

Sample	Special treatment	Detection method	Number of circRNAs	References
Cell line (HeLa)	Pol II CLIP	RNA-seq	15 EIciRNAs (most abundant)	[16]
39 ENCODE data sets	rRNA depletion	RNA-seq	7112 predicted circRNAs (circRNA fraction ≥10%)	[15]
Cell line (H9)	poly(A) RNA depletion rRNA depletion RNase R	RNA-seq	103 ciRNAs (at least 2-fold enrichment)	[14]
Cell line (Hs68)	rRNA depletion RNase R	RNA-seq	25,166 predicted circRNAs (high-confidence)	[12]
15 Cell lines (including cancer and non-cancer cell lines from public ENCODE RNA-seq data)	poly(A) RNA depletion rRNA depletion	RNA-seq	46,866 predicted circRNAs (at an FDR of 0.025)	[11]
4 Cell lines ($CD19^+$ leukocytes, HEK293, $CD34^+$ leukocytes, neutrophils)	rRNA depletion	RNA-seq	1950 predicted circRNAs (at least two independent reads)	[13]
5 Cell lines ($CD19^+$ leukocytes, HeLa, H9, $CD34^+$ leukocytes, neutrophils)	rRNA depletion	RNA-seq	2748 predicted circRNAs	[10]

Special treatments were conducted after total RNAs were extracted from the samples. Then, circRNAs were identified via RNA-seq.
circRNAs: circular RNAs; EIciRNAs: exon–intron circRNAs; ciRNAs: circular intronic RNAs; rRNA: ribosomal RNA; RNA-seq: RNA-sequencing; Pol II CLIP: RNA polymerase II crosslinking and immunoprecipitation; FDR: false discovery rate; RNase R: ribonuclease R.

unlikely to play critical roles in biological processes. Until 2010, few circRNAs had been discovered, and research into circRNA biogenesis was minimal. However, with the development of high-throughput sequencing technology and computational analysis, thousands of circRNAs across species from Archaea to humans have been discovered [10–16,38]. The expression of some circRNAs is >10-fold higher than those of their canonical linear transcripts of the same genes [12]. The recently identified human circRNAs are depicted in Table 1.

Biogenesis of circRNAs

Recent studies have revealed that the biogenesis of circRNAs via backsplicing is different from the canonical splicing of linear RNAs [18]. Furthermore, several recent advances in our understanding of circRNA biogenesis, particularly regarding its regulation and the competition between backsplicing and canonical splicing, have been made [1]. For example, Jeck et al. put forward two models of circRNA formation [12]. Model 1 is termed 'lariat-driven circularization' or 'exon skipping' (Fig. 1a), and model 2 is termed 'intron-pairing-driven circularization' or 'direct backsplicing' (Fig. 1b). Notably, Kelly and colleagues also found that exon circularization is widespread and correlated with exon skipping in human umbilical vein endothelial cells (HUVECs) treated with tumor necrosis factor α (TNFα) or tumor growth factor β (TGFβ) [22]. Although some evidence has indicated that intron-pairing-driven circularization might occur more frequently than lariat-driven circularization [39], accumulated evidence has verified the model of intron-pairing-driven circularization and suggested that reverse complementary sequences, including IRAlus, are important for circRNA biogenesis [17–21,23–25]. Shortly thereafter, Zhang and others discovered a new type of circRNA in human cells that is derived from introns and was termed circular intronic RNAs (ciRNAs). ciRNA biogenesis depends on a consensus motif containing a 7-nt GU-rich element near the 5′ splice site and an 11-nt C-rich element near the branchpoint site [14] (Fig. 1c). Very recently, Li et al. also found exons that are circularized with introns 'retained' between the exons. These authors termed them exon–intron circRNAs or EIciRNAs and found that they could be overexpressed with their flanking complementary sequences [16]. However, the mechanism of EIciRNA formation remains unknown. These mechanisms add considerably to the regulatory complexity of the human transcriptome.

Additionally, researchers have identified the *muscleblind* protein (MBL), which can bind to circMbl flanking introns to provoke the formation of circRNAs that act as RBPs to bridge two flanking introns close together [23]. Similarly, researchers reported an additional mode of circRNA biogenesis in which interactions between RBPs form a bridge between the flanking introns, which causes the splice donor and splice acceptor to close to promote circRNA biogenesis [40] (Fig. 1d). Surprisingly, Conn and others have recently found that RBP Quaking (QKI) regulates the formation of circRNAs [26]. In contrast, Ivanov and others noted that the RNA-editing enzyme ADAR1 can bind to double-stranded RNA to antagonize circRNA biogenesis by melting the stem structure [20]. Therefore, RBPs may serve as activators or inhibitors of the formation of circRNAs in some conditions.

Remarkably, Zhang et al. first proposed a model of alternative circularization that is similar to alternative splicing [18] (Fig. 2). These authors found that competition in RNA pairing by complementary sequences (either repetitive or nonrepetitive) across or within individual flanking introns could significantly affect splicing selection and exon circularization. Complementary sequences within individual flanking introns can be sufficient to promote liner mRNA generation. Conversely, complementary sequences across flanking introns can benefit exon circularization. The competition between reverse complementary sequences can result in multiple circRNA transcripts being processed from a single gene (Fig. 2). However, alternative circularization can be species-specific due to the different distributions of complementary sequences across species. The existence of complementary sequences is necessary but not sufficient for exon circularization [18]. This model suggests that the mechanism of alternative circularization is very complicated and is also possibly regulated by other factors, such as RBPs [1].

Properties of circRNAs

According to recent research, there are several noteworthy properties of circRNAs that are produced by backsplicing. Firstly, these circRNAs have covalently closed loop structures with neither 5′–3′ polarity nor a polyadenylated tail, which makes them much more stable than liner RNA and insusceptible to degradation by RNA exonuclease or RNase R [41]. For example, researchers identified >400 circRNAs in human cell-free saliva (CFS) from healthy individuals. These data represent experimental validation of circRNAs in any type of extracellular body fluid [33]. Secondly, there is a great diversity of circRNAs [40]. In some cases, the abundance of circular molecules exceeds those of the corresponding linear mRNAs by >10-fold [12]. Thirdly, circRNAs are largely composed of exons, which primarily reside in the cytoplasm and possibly have miRNA response elements (MREs) [11–13]. Moreover, circRNAs harbor significant reductions in polymorphisms at predicted miRNA target sites [42]. Some circRNAs come from introns or exons with introns that are 'retained' between exons and are primarily located in the nucleus in eukaryotes and may regulate gene expression [14,16].