Pharmacology & Therapeutics xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Circular RNA and its mechanisms in disease: From the bench to the clinic

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ARTICLE INFO

Keywords:
circRNAs
Biogenesis
Function
Disease
Biomarker
Therapeutic target

ABSTRACT

The emerging recognition of the functional roles of circular RNAs (circRNAs) has given rise to a new perspective regarding our understanding of cellular physiology and disease pathogenesis. Unlike linear RNAs, circRNAs are covalently closed continuous loops that act as gene regulators in mammals, and their sequence composition determines the mode of circRNA biogenesis. The availability and integrated use of advanced genome analysis platforms have allowed the identification of a large number of these molecules. Their high abundance, stability and evolutionary conservation among species endow circRNAs with numerous potential functions, such as acting as microRNA (miRNA) sponges or binding to RNA-associated proteins to form RNA-protein complexes that regulate gene transcription. Moreover, circRNAs have been shown to be expressed in a tissue-specific manner and in pathological conditions, which has stimulated significant interest in their role in human disease and cancer. In this concise review, we outline the characteristics, functions and mechanisms of action of circRNAs as well as their involvement in different diseases. Although their exact roles and mechanisms of gene regulation remain to be clarified, circRNAs have potential applications as disease biomarkers and novel therapeutic targets.

Contents

Abbreviations: circRNAs, circular RNAs; miRNAs, microRNAs; RBPs, RNA-binding proteins; pre-mRNAs, precursor mRNAs; ecircRNAs, exonic circular RNAs; ciRNAs, circular intronic RNAs; ElciRNAs, exon-intron circular RNAs; tricRNA, tRNA intronic circular RNA; MBL, Muscleblind; QKI, quaking; ADAR1, adenosine deaminase acting on RNA 1; U1 snRNP, U1 small nuclear ribonucleoprotein; Pol II, RNA polymerase II; ceRNAs, endogenous RNAs; AD, Alzheimer's disease; FUS, fused in sarcoma; NP, neuropathic pain; MSA, multiple system atrophy; LPS, lipopolysaccharide; MI, myocardial infarction; GC, gastric cancer; CRC, colorectal cancer; HCC, hepatocellular carcinoma; OSCC, oral squamous cell carcinoma; ESCC, esophageal squamous cell carcinoma; LAC, lung adenocarcinoma; ccRCC, clear cell renal cell carcinoma; PCa, prostate cancer; HDFs, human dermal fibroblasts; NASH, nonalcoholic steatohepatitis; HSCR, Hirschsprung's disease; PE, preeclampsia; OA, osteoarthritis; ECM, extracellular matrix; CTEPH, chronic thromboembolic pulmonary hypertension.

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https://doi.org/10.1016/j.pharmthera.2018.01.010
0163-7258/

Please cite this article as: Han, B., et al., Circular RNA and its mechanisms in disease: From the bench to the clinic, *Pharmacology & Therapeutics* (2018), https://doi.org/10.1016/j.pharmthera.2018.01.010

1. Introduction

Protein-coding genes and their transcripts are the most studied sequences in eukaryotic cells (Esteller, 2011). However, protein-coding genes and RNAs comprise only a small fraction of genomes and transcriptomes (Alexander, Fang, Rozowsky, Snyder, & Gerstein, 2010). Indeed, the vast majority of sequences in the human genome do not encode proteins, and non-coding RNAs account for almost 95% of the total RNA transcribed from eukaryotic genomes (Warner, 1999). Non-cording RNAs, which are largely classified as transcribed ultraconserved regions, microRNAs (miRNAs), small nucleolar RNAs, PIWI-interacting RNAs, long non-cording RNAs, and circular RNAs (circRNAs), are being increasingly recognized as functioning in gene regulation and contributing to the development of many human disorders (Esteller, 2011; Mercer, Dinger, & Mattick, 2009). As a large proportion of the non-coding RNA family, circRNAs have drawn intense interest over the last few years. Unlike linear RNA molecules, circRNAs are closed circular molecules with a covalently closed loop structure that lack 5′-3′ polarity or a polyadenylated tail (Chen & Yang, 2015).

circRNAs were first identified in 1976 in an electron microscopy-based study of RNA viruses (Sanger, Klotz, Riesner, Gross, & Kleinschmidt, 1976) and have since been found in humans, mice, rats, fungi and other organisms (Capel et al., 1993; Cocquerelle, Daubersies, Majerus, Kerckaert, & Bailleul, 1992; Kolakofsky, 1976; Matsumoto, Fishel, & Wickner, 1990; Zaphiropoulos, 1996, 1997). Nonetheless, due to the lack of reliable high-throughput detection methods, only a handful of circRNAs have been identified in the past 30 years (Capel et al., 1993; Cocquerelle et al., 1992; Cocquerelle, Mascrez, Hetuin, & Bailleul, 1993; Kos, Dijkema, Arnberg, van der Meide, & Schellekens, 1986; Nigro et al., 1991; Zaphiropoulos, 1993, 1996). Based on their structural specificity, unknown functions and low abundance (Nigro et al., 1991), circRNAs were initially considered ancient and conserved molecules produced as errant byproducts of splicing and did not receive much attention.

However, advances in biotechnology, particularly bioinformatics and high-throughput sequencing technology have resulted in the discovery and identification of a large number of circRNAs. Indeed, circRNAs are abundant, diverse and conserved molecules that are often expressed in a tissue- and developmental stage-specific manner (Jeck et al., 2013; Rybak-Wolf et al., 2015; Salzman, Chen, Olsen, Wang, & Brown, 2013). Our knowledge regarding their functions has also expanded with their identification. Specifically, circRNAs might function as miRNA sponges to prevent mRNA translation (Hansen et al., 2013; Wang, Long, et al., 2016) and influence gene expression by regulating splicing (Ashwal-Fluss et al., 2014; Li, Huang, et al., 2015) or transcription and by interacting with RNA-binding proteins (RBPs) (Du et al., 2016). circRNAs play a critical role in biological processes and are reported to participate in multiple processes involved in disease progression, and thus, these molecules offer new potential opportunities for therapeutic intervention and might serve as diagnostic biomarkers. In this review, we briefly introduce the biogenesis, characteristics and function of circRNAs and highlight their roles in different diseases. Considering their ubiquitous presence and diversity, circRNAs might be major contributors to normal cellular physiological or pathological processes.

2. Characteristics of circRNAs

2.1. Broad presence and expression

Through analyses of transcriptome sequencing datasets with computational pipelines that specifically search for back-splicing junctions, the expression of circRNAs has been widely detected in a large number of metazoans and in diverse cell types and organisms, ranging from fruit flies to humans (Ivanov et al., 2015; Westholm et al., 2014; Zheng et al., 2016). circRNAs have also been found in plants (Lu et al., 2015; Sun et al., 2016; Ye, Chen, Liu, Zhu, & Fan, 2015) and other organisms, such as protists (Broadbent et al., 2015) and fungi (Wang et al., 2014). A total of 5.8% to 23% of actively transcribed human genes reportedly produce circRNAs (Conn et al., 2015; Kelly, Greenman, Cook, & Papantonis, 2015), and these circRNAs are dynamically regulated among tissues and cell types (Du et al., 2016; Li, Chen, et al., 2015). Although one study suggested that the levels of circRNAs and their linear counterparts are not highly correlated (Salzman et al., 2013), others have reported that expression changes between circRNAs and linear variants from the same gene are in fact largely correlated (Hansen et al., 2013; Jeck et al., 2013; Salzman, Gawad, Wang, Lacayo, & Brown, 2012). The potential functions of circRNAs are consistent with their widespread and regulated expression.

2.2. Stability

Due to their resistance to RNA exonucleases or RNase R, circRNAs are more stable than linear RNAs (Jeck et al., 2013; Suzuki et al., 2006), which might lead to the accumulation of circRNAs and thus a higher concentration of circRNAs than linear RNAs in quiescent and post-mitotic cells, such as neurons (Chen & Schuman, 2016). circRNAs also accumulate in some physiological processes, such as neuronal differentiation, fetal development and synaptic development (Rybak-Wolf et al., 2015; Szabo et al., 2015; Westholm et al., 2014; You et al., 2015), and this accumulation of circRNAs indicates that they might function in these processes. Furthermore, due to their high stability in blood and other body fluids, circRNAs are suitable biomarkers for disease diagnosis.

2.3. Conservation

circRNA expression appears to be conserved across mammals (Barrett & Salzman, 2016), and some are even conserved in evolutionarily distant *Drosophila* (Rybak-Wolf et al., 2015). In relatively closely related species, such as humans and mice, 4% of orthologous genes can generate circRNAs (Salzman et al., 2013), and approximately 5–30% of these circRNAs are completely conserved (Guo, Agarwal, Guo, & Bartel, 2014; Jeck et al., 2013; Memczak et al., 2013). In addition, approximately 5–10% of human brain circRNAs are expressed in the porcine brain (Barrett & Salzman, 2016; Veno et al., 2015), and 23% of circRNAs are conserved between mouse and rat (You et al., 2015). Taken together, the findings show that circRNAs are unlikely to be non-functional byproducts.

3. circRNA biogenesis

Although it is known that circRNAs are derived from precursor mRNAs (pre-mRNAs), their biogenesis remains elusive. circRNAs differ from other RNAs in their remarkable continuous closed loop structure, which is covalently linked by free 3′ and 5′ ends (Granados-Riveron & Aquino-Jarquin, 2016). This closed loop structure, which is also called a "back-splicing" structure, is generated from the joining of an upstream 3′ splice site to a downstream 5′ splice site (Barrett & Salzman, 2016). Similar to canonical splicing, the formation of the 'back-splicing' structure requires not only a canonical splicing signal but also the canonical spliceosome machinery (Andres-Leon, Nunez-Torres, & Rojas, 2016). As shown in Fig. 1, several pathways participate in the circularization of circRNAs. circRNAs can mainly be classified in three categories: exonic circular RNAs (ecircRNAs) (Jeck et al., 2013; Memczak et al., 2013; Salzman et al., 2013; Zhang et al., 2013), circular intronic RNAs (ciRNAs) (Zhang et al., 2013), and exon-intron circular RNAs (EIciRNAs) (Li, Huang, et al., 2015). RBP pairing (Ashwal-Fluss et al., 2014; Conn et al., 2015) and intron pairing (Lee & Rio, 2015; Rybak-Wolf et al., 2015; Zhang et al., 2014) drive circularization in the direct back-splicing pathway of circRNA formation. Some RBPs, including Muscleblind (MBL) (Ashwal-Fluss et al., 2014), Quaking (QKI) (Conn et al., 2015) and adenosine deaminase acting on RNA 1 (ADAR1) (Ivanov et al., 2015), participate in the regulation of circRNA biogenesis. MBL and

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