Mechanisms of Ageing and Development 178 (2019) 16–24

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

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

Circular RNA involvement in aging: An emerging player with great potential

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ARTICLE INFO

Keywords:
Circular RNA
miRNA
Aging
Age-related disease
Senescence

ABSTRACT

Circular RNA (circRNA) is a class of newly discovered noncoding RNA (ncRNA), presenting as a special covalent loop without a 5′ cap or 3′ tail. Multiple biological properties of circRNA have been revealed during the past decades, such as widespread expression, high conservation, cell-specificity, tissue-specificity and developmental stage-specific expression patterns, as well as resistance to RNase R digestion. CircRNA also exhibits diverse biological functions, including regulation of host genes, alternative splicing, miRNA sponges, protein traps and even protein synthesis. Recently, a global accumulation of circRNAs during aging has been identified across different species, indicating a potential role as a causal factor in aging and age-related disease. The high stability could be one of the mechanisms contributing to this phenomenon. CircRNA could play a role in aging, such as neural aging, muscle aging, reproductive aging, skin aging, immunosenescence, visual aging and age-related diseases like Alzheimer's disease, via interaction with miRNAs, RNA binding proteins, modulation of parental gene transcription, mainly at the transcriptional and posttranscriptional levels. The present study will focus on the advancement of circRNA regarding aging and age-related diseases. We will also discuss the biogenesis, properties, biological functions, and the perspectives of circRNA.

1. Introduction

Circular RNA (circRNA) is a novel class of noncoding RNA (ncRNA) of approximately 100 nucleotides (nt) in length, with covalently closed loop structures but without 5′ caps or 3′ poly (A) tails. In 1976, they were first discovered in viroids as byproducts from aberrant splicing (Cocquerelle et al., 1993; Sanger et al., 1976). Due to their low abundance and unknown biological functions, they did not receive much attention at the time. In recent years, with the advent of next-generation RNA sequencing (RNA-seq) and bioinformatics approaches, several circRNAs have been discovered. It has been confirmed that circRNAs exhibit biological features, such as broad expression in cells and tissues, cell-, tissue- and developmental stage-specific expression, resistance to RNase R digestion, and highly evolutionary conservation (Chen, 2016; Ivanov et al., 2015; Jeck et al., 2013). Additionally, circRNA is reported to perform multiple regulatory functions, including the modulation of parental gene transcription (Li et al., 2015b), acting as microRNA (miRNA) sponges (Hansen et al., 2013) and interacting with RNA-binding proteins (RBPs) (Conn et al., 2015), mainly at the transcriptional and posttranscriptional levels. More excitingly, some circRNAs are proven to have protein-coding potential, thus expanding the eukaryotic proteome (Legnini et al., 2017; Pamudurti et al., 2017; Yang et al., 2017).

Currently, numerous studies have reported circRNAs in cardiovascular diseases, diabetes, cancer and neurological disorders, indicating their pivotal roles in the pathogenesis of human diseases and their potential as biomarkers for the diagnosis and prognosis of many human diseases (Jiang et al., 2017; Kumar et al., 2017; Shan et al., 2017; Zhou et al., 2018). Recently, it has been demonstrated that circRNA plays a role in both cellular senescence and cellular survival (Maiese, 2016). From a wide range of organisms, including nematodes (Cortés-López et al., 2018), fruit flies (Hall et al., 2017), mice (Gruner et al., 2016), rats (Wang et al., 2018), monkeys (Abdelmohsen et al., 2015), tree shrews (Lu et al., 2018), and porcine (Chen et al., 2018a), researchers have discovered a large number of circRNAs differentially expressed during aging, suggesting their possible involvement in aging and age-related diseases. On one hand, circRNA may prevent cellular proliferation by binding to the cell cycle proteins cyclin-dependent kinase 2 (CDK2) and cyclin-dependent kinase inhibitor 1 (p21), as well as through mammalian forkhead transcription factors (Du et al., 2017, 2016). On the other hand, circRNA also oversees cellular survival through programmed cell death involving apoptosis (Holdt et al., 2016; Wang et al., 2016). Nevertheless, the underlying mechanisms of circRNA involvement in the regulation of aging and age-related diseases

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https://doi.org/10.1016/j.mad.2018.11.002
Received 5 September 2018; Received in revised form 25 October 2018; Accepted 30 November 2018

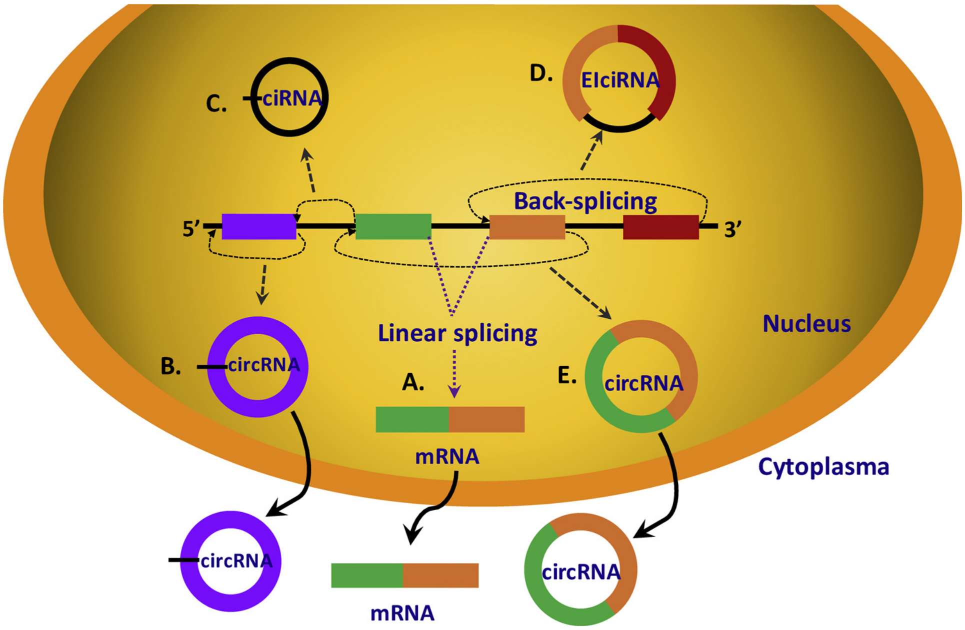

Fig. 1. The biogenesis and subcellular localization of circRNAs. A. Canonical linear splicing of mRNA. The adjacent exons will form a linear mRNA after removing the introns. B and E. Exon-derived circRNA originating from back-splicing often contains one or more exon(s); C. Intronic circRNA (ciRNA) by back-splicing consists of only intron(s); D. Exon-intron circRNA (EIciRNA) is generated via back-splicing without removing the intron(s). ciRNA and EIciRNA both locate in the nucleus, while circRNA migrates to the cytoplasm.

remain to be elucidated.

In this review, we will briefly discuss the known properties and functions of circRNA before specifying our primary focus regarding recent progress of circRNA in aging and age-related diseases in order to provide new targets and ideas for the study of aging.

2. Categories and biogenesis of circRNAs

According to the locations of their origin sequences in the genome, circRNA molecules can be classified into three subtypes (Fig. 1): exonic circRNA (ecircRNA) (Jeck et al., 2013), intronic circRNA (ciRNA) (Zhang et al., 2013) and exon-intron circRNA (EIciRNA) (Li et al., 2015b; Salzman et al., 2013). Commonly, most ecircRNAs tend to be cytoplasmic (Jeck et al., 2013; Memczak et al., 2013), while ciRNAs and EIciRNAs are predominantly located in the nucleus, indicating their potential roles in parental gene regulation (Li et al., 2015b; Zhang et al., 2013). Unlike the canonical splicing of linear RNA, the biogenesis of circRNA occurs through back-splicing (Zhang et al., 2014a). In 2013, Jeck et al. (2013) first proposed two classical models of circRNA biogenesis: lariat-driven circularization and intron-pairing-driven circularization. Shortly thereafter, Zhang et al. (2014a) proposed a model of alternative circularization, in which complementary sequences across or within individual flanking introns could significantly influence splicing selection and exon circularization. However, due to the different distributions of complementary sequences across species, alternative circularization can be species-specific (Zhang et al., 2014a). Another model of circRNA biogenesis was then introduced. By binding with RNA-binding proteins (RBPs), a bridge between the flanking introns is formed, bringing the splice donor and acceptor closer together, thereby promoting the circularization of exons (Ashwal-Fluss et al., 2014; Conn et al., 2015). Several splicing factors/RBPs, including Muscleblind, Quaking (QKI), NF90/NF110 (RNA-binding domain containing immune factors), hnRNPs, and SR proteins, have been proven to regulate circRNA biogenesis (Ashwal-Fluss et al., 2014; Conn et al., 2015; Kramer et al., 2015; Li et al., 2017). Furthermore, RNA-Binding Motif protein 20 (RBM20) has been recognized as a new splicing factor that was recently implicated in circRNA production, specifically from the *TTN* gene (Aufiero et al., 2018; Khan et al., 2016).

3. Properties of circRNAs

Based on current research, several remarkable properties of circRNA have emerged. First, given that circRNA molecules are covalently closed loop structures without 5′ caps and 3′ poly(A) tails, they are much more stable than are linear RNAs and they demonstrate resistance to RNase R digestion (Suzuki and Tsukahara, 2014). Second, circRNAs are widespread in cells and tissues. According to some studies, the expression of certain circRNAs are more than ten times higher than those of their canonical linear transcripts generated from the same genes (Jeck et al., 2013). Third, circRNAs often exhibit cell-, tissue- and developmental stage-specific expression patterns (Li et al., 2015b; Memczak et al., 2013; Salzman et al., 2013), indicating that they may be involved in physiological and pathological conditions. Fourth, circRNAs are highly evolutionary conserved at the sequence level among different species (Jeck et al., 2013). For example, 28% of circRNA molecules in mice are conserved in humans (Rybak-Wolf et al., 2015). Moreover, the results from three different *Drosophila* species revealed that circRNAs among divergent species are broadly conserved, and some circRNA detected in fly brains are also expressed in mammalian brains (Westholm et al., 2014). Finally, some circRNAs harbor internal ribosome entry sites (IRESs), which provide the potential to synthesize proteins (Legnini et al., 2017; Pamudurti et al., 2017; Yang et al., 2017). Taken together, these properties indicate that circRNAs are potential regulators of transcription and posttranscription and may participate in the development of human diseases.

4. Biological functions of circRNAs

To date, although hundreds of thousands of circRNAs have been identified among different species, the biological functions of most circRNAs remain unclear. Several studies have revealed diverse biological functions of circRNAs, including regulation of parental gene expression (Li et al., 2015a, b; Zhang et al., 2013), binding to RBPs or other proteins (Ashwal-Fluss et al., 2014; Du et al., 2016; Li et al., 2015b), acting as miRNA sponges (Hansen et al., 2013; Tao et al., 2017; Thomas and Saetrom, 2014; Wang et al., 2016) and translating into proteins (Legnini et al., 2017; Pamudurti et al., 2017; Yang et al., 2017). This implies that circRNA may regulate gene expression at multiple levels (see Fig. 2).

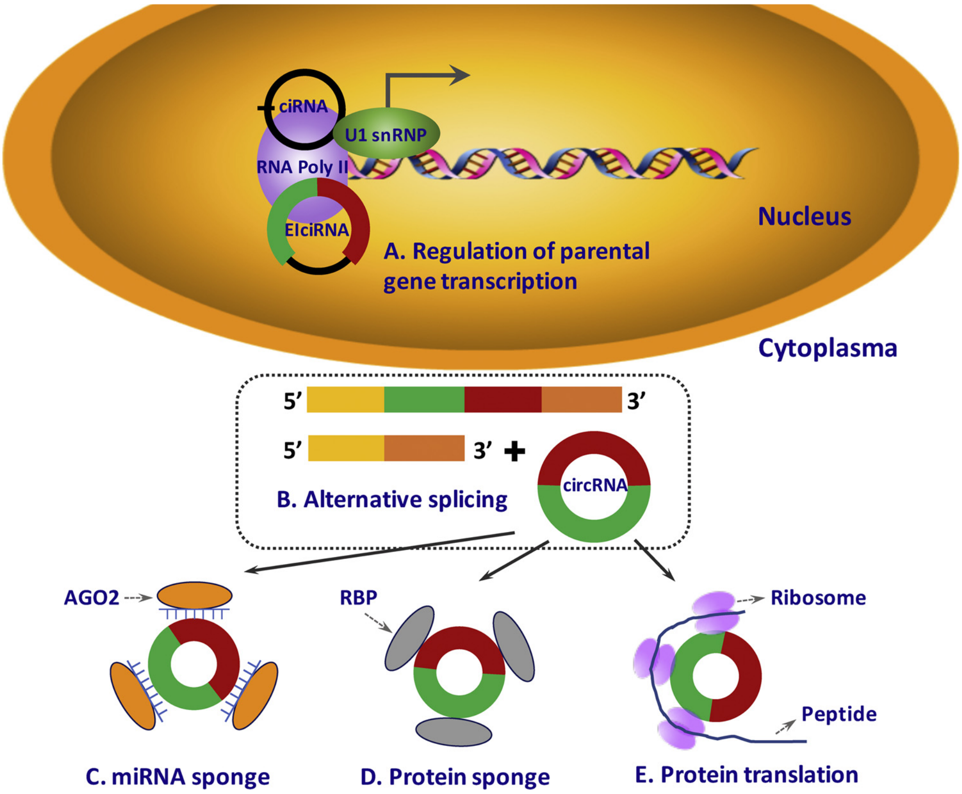

Fig. 2. The putative biological functions of circRNA. A. Regulation of parental gene transcription: the ciRNA and EIciRNA are located in the nucleus and are involved in their parental gene expression. B. Alternative splicing: the biogenesis of circRNA is generally accompanied with the transcription of their parental genes. Therefore, circRNA may competitively influence the biogenesis and processing of mRNA. C. miRNA sponge: some circRNAs have conserved binding sites for miRNAs. Through competitive binding with miRNAs, these circRNAs can block the binding between miRNAs and their target mRNAs, subsequently prohibiting the repressive effects of miRNAs on the target protein translation. D. Protein sponge: to regulate the function of RBPs, some circRNAs can interact with RBPs, such as Argonaute, polymerase II, muscleblind, and others. E. Protein translation: some circRNAs contain an internal ribosome entry site that can bind with ribosomes.

4.1. *Regulation of parental gene transcription*

Studies have shown that circRNAs take part in regulation of expression of parental genes (Li et al., 2015a, b; Zhang et al., 2013). For example, ciRNAs are generated from lariat introns in the nucleus that escape from debranching (Zhang et al., 2014b). Researchers have found that stable ciRNA binds to RNA polymerase II (Pol II) and modulates host gene transcription in a *cis*-acting manner (Zhang et al., 2013). Furthermore, EIciRNA binds to U1 small nuclear ribonucleoproteins (snRNP) through a specific RNA–RNA interaction, and then the EIciRNA–U1 snRNP complexes might interact with RNA Pol II transcription complex to promote host gene expression (Li et al., 2015b). According to a previous study, *circ-ITCH* shares some miRNA binding sites with 3′-untranslated regions (3′-UTR) of the transcript from their parental gene *ITCH*. In this way, interactions of *circ-ITCH* with miR-7, miR-17 and miR-214 might increase the level of *ITCH* (Li et al., 2015a). Abdelmohsen and colleagues also discovered that *circPABPN1* prevented HuR binding to *PABPN1* mRNA and lowered *PABPN1* translation (Abdelmohsen et al., 2017).

4.2. *Alternative splicing*

Recent advances have suggested that circRNA could regulate alternative splicing. Circularization and canonical pre-mRNA splicing can compete against one another, thus enabling ecircRNAs to play a role in alternative splicing (Ashwal-Fluss et al., 2014). In general, once an exon is circularized, the less it will be involved in the production of linear mRNA (Kelly et al., 2015). It is reported that circRNA production is positively correlated with exon skipping, which is the most common alternative splicing event in human pre-mRNA (Kelly et al., 2015). However, not all skipped exons could generate circRNA, implying that other factors might be involved. A recent study found that cardiac circRNAs were primarily derived from constitutive exons, indicating that these circRNAs were produced at the cost of their linear transcripts (Aufiero et al., 2018). Therefore, the production of circRNA could impact the accumulation of the linear mRNA, and thereby, could fine-tune the gene expression. More studies are needed to further illuminate the underlying mechanisms.

4.3. *miRNA sponge*

miRNAs are abundant short ncRNA molecules of 22 nt that post-transcriptionally regulate gene expression via binding to complementary sequences on target mRNAs (Hansen et al., 2013). circRNAs have been determined to contain miRNA response elements (MREs), which might act as competing endogenous RNAs (ceRNAs) to prevent miRNAs from binding to their target genes. Thus, given the presence of miRNA binding sites, circRNA can indirectly affect the expression of mRNAs downstream (Memczak et al., 2013). *CDR1as* (*ciRS*-7), which contains 74 binding sites for miR-7, inhibits the action of miR-7 by binding to Argonaute protein 2 (AGO2), thereby increasing the expression level of the miRNA-7 target genes (Hansen et al., 2011). Likewise, the Sex-determining region Y (*Sry*) was reported to produce a testis-specific circular transcript, acting as a miR-138 sponge in HEK293 cells (Hansen et al., 2011). Recently, bioinformatics analysis has demonstrated that tens of thousands of circRNAs have miRNA adsorption function, although only a few of them have been validated (Thomas and Saetrom, 2014). Therefore, the biological function of the miRNA sponge requires further elucidation.

4.4. *Protein sponge*

It has been reported that RBPs harbor domains for RNA recognition and binding, thus playing indispensable roles in RNA dynamics. The phenomenon of protein adsorption can also be seen in circRNA. For example, *circFoxo3*, which is derived from a tumor suppressor gene, arrests the function of CDK2, a cell cycle protein, and blocks cell cycle progression via formation of a ternary complex (Du et al., 2016). By specific interaction with U1 snRNP to form a complex, *circRIF3J* (an EIciRNA) enhances the parental gene transcription via binding to RNA

Pol II (Li et al., 2015b). It has been proven that *circMbl*, which is derived from exon 2 of *MBNL1* (or *muscleblind*) in humans and flies, can directly bind to muscleblind protein. Furthermore, muscleblind can in turn interact with the flanking intron to regulate the formation of *circMbl* (Ashwal-Fluss et al., 2014). Other lines of evidence have clarified the interaction between circRNAs and various RBPs, such as QKI (Conn et al., 2015), Argonaute (Hansen et al., 2013), IPM3 (Schneider et al., 2016) and others. Taken together, circRNAs serve as scaffolds between proteins and nucleic acids to achieve certain biological functions.

4.5. Protein translation

As one of the ncRNA members, circRNAs were thought to possess no protein-coding potential. This was probably due to their lack of both a 5′ 7-methylguanosine cap structure and a poly(A) tail, two features that are required for efficient translation of linear mRNA (Jackson et al., 2010). However, several studies have reported that exon-derived circRNAs contain translation initiation sites, which can initiate translation of engineered circRNAs in vitro, in *E. coli*, in eukaryotes, or human cells using a rolling circle amplification mechanism (Abe et al., 2015; Chen and Sarnow, 1995; Perriman et al., 1998; Wang and Wang, 2015). In 2017, Yang et al. (2017) first discovered that consensus *N6*-methyladenosine (m6A) motifs were enriched in many circRNAs and a single m6A site was sufficient to drive translation initiation. Subsequently, Legnini et al. (2017) and Pamudurti et al. (2017) demonstrated that endogenous circRNAs provided the potential for generation of proteins, thereby expanding the eukaryotic proteome and revealing novel modes of cap-independent translation. However, further studies of the translation mechanisms of circRNA remain warranted.

5. CircRNAs as promising biomarkers for human diseases

Currently, clinicians are trying to seek a minimal-invasive method for early detection of human diseases. CircRNAs are enriched and stable in body fluid such as peripheral blood (serum or plasma), urine, saliva, cerebrospinal fluid, and free-floating cells like circulating blood cells, tumor cells, and exosomes (Zhang et al., 2018). Additionally, compared with standard biomarkers, the special biological properties of circRNAs provides with better stability and flexible storage conditions for the samples, and elevated sensitivity and specificity (Ewa et al., 2018). Therefore, these ncRNAs are considered to be suitable biomarkers for liquid biopsies.

So far, circRNAs as biomarkers for cancer, like gastric cancer (GC), hepatocellular carcinoma (HCC), lung cancer, *etc*., have been extensively investigated. For instance, *circPVT1* upregulated in GC tissues was found to be an independent prognostic biomarker for survival of patients with GC (Chen et al., 2017). Similarly, *circZKSCAN1* and *circITCH* were proved to serve as diagnostic biomarkers of HCC (Yao et al., 2017; Guo et al., 2017). Besides, some circRNAs also show potentials to be biomarker candidates for age-related diseases, like cardiovascular diseases, neurodegenerative disorders, and other human diseases. Zhao et al. (2017) reported that hsa_circ_0124644 was identified as a biomarker for coronary artery disease in peripheral blood with a sensitivity and specificity of 0.861and 0.626, respectively. And *circANKRD36* may be involved in type 2 diabetes mellitus (T2DM) and inflammation-associated pathways via interaction with miRNAs (hsa-miR-3614-3p, hsa-miR-498, and hsa-miR-501-5p) and can be used as a potential biomarker (Fang et al., 2018). Collectively, circRNAs have been proved as putative biomarkers for the diagnosis and prognosis of human diseases.

6. CircRNA in aging and age-related diseases

Aging is the gradual decline of cells, tissues and organs, which is commonly characterized by a decline in muscle mass, reduced blood flow, impaired immune recognition, reduced energy utilization, cognitive impairment and a decline in stress-response capability, thus leading to the occurrence of age-related diseases, such as cardiovascular diseases, neurodegeneration, obesity, diabetes, and others (Grammatikakis et al., 2014). López-Otín et al. (2013) summarized the molecular hallmarks of aging for the first time: genomic instability, telomere loss, epigenetic changes, protein loss, malnutrition, mitochondrial dysfunction, cell senescence, stem cell depletion and intercellular communication changes. ncRNAs can regulate gene expression at transcriptional, post-transcriptional and posttranslational levels, and they provide important regulatory roles for cellular processes, such as proliferation, differentiation, resting, and immune responses (Singh and Prasanth, 2013; Yoon et al., 2013). There have been many studies concerning the expression of circRNAs in cardiovascular diseases (Burd et al., 2010; Du et al., 2017; Li et al., 2018; Wang et al., 2016; Wu et al., 2017), diabetes (Jiang et al., 2017; Shan et al., 2017; Yan et al., 2018), cancer (Han et al., 2017; Yu et al., 2018; Zhang et al., 2017b) and neurological diseases (Floris et al., 2017; Lukiw, 2013), indicating their potential roles in the pathogenesis of human diseases. Recent studies have shown that circRNA could modulate cell senescence and cell survival and may be involved in the regulation of aging and age-related diseases (Maiese, 2016; Panda et al., 2017; Knupp and Miura, 2018). Thus, circRNA is expected to be a new biological marker and target for aging and age-related diseases (Table 1).

6.1. CircRNA in neural aging and Alzheimer's disease

Recent studies have demonstrated that circRNAs are highly abundant, conserved and are dynamically expressed in the mammalian brain (Rybak-Wolf et al., 2015). CircRNAs are generally upregulated during neuronal differentiation, highly enriched in synaptoneurosomes and are often differentially expressed, irrespective of their mRNA isoforms. It is reported that nearly 20% of the protein-coding genes in mammalian brains can generate circRNAs at the expense of the corresponding linear transcripts, indicating that the biogenesis of circRNA could be an important regulator for mRNA synthesis (Rybak-Wolf et al., 2015). The functions of circRNA in the brain have been summarized as follows (Hanan et al., 2017): (i) serving as "memory" molecules under stressful insults; (ii) serving as templates for protein translation in the cytoplasm or in synapses; (iii) acting as miRNA sponges; (iv) active transport to the synapse or dendrites, and serving as transport-hubs for RBPs; and (v) encoding information that can be stored or even passed on between cells.

Westholm et al. (2014) first identified numerous circRNA molecules in the *Drosophila* brain, most of which were conserved across multiple *Drosophila* species. Notably, these circRNAs harbored numerous well-conserved miRNA binding sites, indicating their potential functions by interacting with miRNA. Intriguingly, several circRNAs accumulated dramatically in neural subcellular compartments (e.g., synapse) during neural aging compared to their corresponding mRNAs, which might serve as a novel class of aging biomarkers. Subsequently, this phenomenon of age-dependent neural accumulation of circRNAs was also discovered among mice (Gruner et al., 2016), *C. elegans* (Cortés-López et al., 2018), and the tree shrew (Lu et al., 2018). The high composition of postmitotic cells in neural tissues, coupled with the high stability of circRNA might together be attributed to this phenomenon (Cortés-López et al., 2018). Additionally, the dysfunction of alternative splicing during the aging process could probably add to the increase of circRNA biosynthesis in the nervous system (Cortés-López and Miura, 2016). Bioinformatics analysis indicated that ubiquitin-mediated proteolysis, the MAPK signaling pathway, and the phosphatidylinositol pathway were closely related to neural aging. An interaction regulatory network exhibited that novel_circRNA_007362 could regulate the expression of UBE4B via adsorption of 24 miRNAs, thus participating in the regulation of neuronal apoptosis (Lu et al., 2018).

Given that circRNAs are preferentially expressed along neural genes and in neural tissues (Floris et al., 2017), they have been linked to

Table 1

Summary of recent advances about circRNAs in aging and age-related diseases.

Species	No. of participants	Age	Sample	Aging and age-related diseases	Detection methods	Special treatment	Number of circRNAs	References
Drosophila	NM	Diverse developmental stages	Heads	CNS aging	RNA-seq	rRNA-depletion	2,513 circRNAs	Westholm et al. (2014)
Rhesus macaque	24	Young: < 6 yr Middle: 11-17 yr Old: 25-41 yr	Skeletal muscle	Muscular senescence	RNA-seq	RNase H treatment	12,007 circRNAs	Abdelmohsen et al. (2015)
Human	29	Young: 25-35 yr Old: 65 to 94 yr	Intravenous blood	Immuno-senescence	circRNA microarray technology	None	3,734 DE-circRNAs**	Wang et al. (2015)
Mouse	NM	Young: 1 m Old: 22 m	Cortex, hippocampus and heart samples	CNS aging	RNA-seq	rRNA-depletion	6,791 circRNAs containing 675 novel circRNAs	Gruner et al. (2016)
Human	6	Young: ≤30 yr Old: ≥38 yr	Follicular fluid	Reproductive aging	circRNA microarray technology	RNase H treatment	57 DE-circRNAs**	Cheng et al. (2017)
Drosophila	400	10, 20, 25,30, 40 d post-eclosion	Eyes	Visual senescence	RNA-seq	RNase H treatment	1209 circRNAs containing 114 novel circRNAs and 38 DE-circRNAs**	Hall et al. (2017)
Arabidopsis	NM	D4 to D30	Leaves	Plant senescence	RNA-seq	None	168 circRNAs containg 40 novel circRNAs and 41 DE-circRNAs	Liu et al. (2017)
Human	4	Children aged 6-8 yr	Dermal fibroblasts	Skin photo-aging	RNA-seq	UVA irradiation	13,775 circRNAs containing 5482 novel and 29 DE-circRNAs*	Peng et al. (2017)
Mouse (SAMP8 and SAMR1)	6	7 m	Cerebral cortex	AD	Total RNA-seq	None	235 DE-circRNAs	Zhang et al. (2017a)
Porcine	4	Young: 180 d Old: 8 yr	CM and SM (*longissimus dorsi muscle*)	Muscular aging	RNA-seq	rRNA-depletion	26 DE-circRNAs related to CM and 62 DE-circRNAs related to SM**	Chen et al. (2018)
C. elegans	12	D0 (L4), D1, D7, D10	Whole worms	Aging	Total RNA-seq	rRNA-depletion	1,166 circRNAs containing 575 novel circRNAs	Cortés-López et al. (2018)
Mouse (SAMP8 and SAMR1)	3	Young: 5 m Old: 8 m	Hippocampus	AD	circRNA microarray technology	None	231 DE-circRNAs*	Huang et al. (2018)
Human	20	AD Healthy elderly control	Astrocytes microdissected from the posterior cingulate	AD	RNA-seq	None	4438 circRNAs	Wang et al. (2018)
Tree shrew (*Tupaia belangeri*)	9	I: 47-52 d Y: 15-18 m O: 78-86 m	Hippocampus and cerebellum	Brain aging and age-related brain diseases	Total RNA-seq	rRNA-depletion	35,007 circRNAs (all were novel)	Lu et al. (2018)
Rat (male Sprague Dawley)	20	AD Sham	Hippocampus	AD	Microarray analysis	Nonw	555 DE-circRNAs	Sekar et al. (2018)

Notes: AD: Alzheimer's disease; CM: cardiac muscle; CNS: central nervous system; d: day; m: month; DE: differentially expressed; RNA-seq: RNA sequencing; SAMR1: senescence-accelerated mouse resistant 1; SAMP8: senescence-accelerated mouse prone 8; yr: year; *: fold change, FC > 1.5, $P < 0.05$; **: FC > 2, $P < 0.05$.

neurological diseases such as Alzheimer's disease (AD), one of the most common neurodegenerative diseases (Burns and Iliffe, 2009). Although many scientists have attempted to explore the origins of AD, the underlying mechanism is not fully understood. Recent studies have identified numerous circRNAs expressed in the neural tissues of AD mice models and in the sporadic AD patients (Huang et al., 2018; Sekar et al., 2018; Wang et al., 2018; Zhang et al., 2017a). Using a senescence-accelerated mouse prone 8 (SAMP8) model (cerebral cortex) which is characterized with an age-related spontaneous deterioration in learning and memory abilities and is often treated as an important sporadic AD model (Kang et al., 2014), researchers (Zhang et al., 2017a) have obtained 235 significantly dysregulated circRNA transcripts. GO analysis exhibited that the axon terminus (GO: 0043679) and synapse (GO: 0045202) were involved in the modulation of AD. Results of circRNA-associated-ceRNA networks demonstrated that Aβ clearance (Hmgb2) and myelin function (Dio2) were closely linked to the regulation of AD. Similarly, Huang and colleagues (Huang et al., 2018) identified some differentially expressed circRNAs in the SAMP8 mice model (hippocampal tissues) compared with the control mice. And among them mmu_circRNA_017963 changed the most significantly. Further bioinformatics analysis signified that mmu_circRNA_017963 was strongly associated with autophagosome assembly, exocytosis, apoptotic process, synaptic vesicle cycle, spliceosome, and glycosaminoglycan in vesicular transport pathways. Similar findings were observed in an Aβ-142-induced AD-like rat model (Wang et al., 2018). A total of 555 significantly dysregulated circRNA were identified in the hippocampus of AD rats. From the results of GO and KEGG pathway analyses, the extracellular region part (GO:0044421), toll-like receptor binding (GO:0035325), regulation of biological quality (GO:0065008), as well as AMPK and p53 signaling pathways were identified, all of which were identical to those previously reported to be involved in AD development (Cai et al., 2012; Merlo et al., 2014). More recently, a total of 4438 unique circRNA were identified in astrocytes microdissected from the posterior cingulate (PC) of sporadic AD patients and healthy elderly controls (Sekar et al., 2018). By constructing circRNA-miRNA-mRNA regulatory networks, researchers found that immune response pathways were significantly enriched in accordance with the function of astrocytes in cerebral innate immunity (Sekar et al., 2018). Collectively, these studies contribute to the understanding of transcriptional regulation in AD brains and provide new insights regarding the diagnosis and therapy of AD in the future.

6.2. CircRNA in muscle aging

Skeletal muscle undergoes dramatic changes with aging, including loss of muscle mass, reduced strength and impaired ability to regenerate, all of which culminates in the development of sarcopenia (Karakelides and Nair, 2005; Tosato et al., 2007). ncRNAs, such as miR-206, miR-29 and miR-431, were proven to be involved in the physiology and pathology of skeletal muscle (Lee et al., 2015; Winbanks et al., 2011). Recently, Abdelmohsen and colleagues have identified ~12,000 circRNAs, including numerous ciRNAs, from monkey skeletal muscle samples (Abdelmohsen et al., 2015). Furthermore, researchers have discovered 19 circRNAs significantly downregulated with aging and speculated that these circRNAs might affect skeletal muscle function.

Aging can significantly affect both cardiac and skeletal muscle, but the aging process of these muscle types may be different. Using high-throughput RNA-seq, Chen et al. (2018a) found that 26 mRNAs, 4 lncRNAs, 22 miRNAs and 26 circRNAs were differentially expressed during cardiac senescence, while 81 mRNAs, 5 lncRNAs, 79 miRNAs and 62 circRNAs were differently expressed during aging of skeletal muscle. Furthermore, they constructed a circRNA-miRNA-mRNA interaction network and found that *circRNA014844*, *circRNA011308*, *and circRNA018401* could regulate the expression of *FGB* (involved in the procoagulation process) during aging via adsorption of miR-493-5p.

Another study has reported that *circFoxo3*, which is derived from the mammalian fork head protein transcription factor Foxo3, was highly expressed in heart specimens of elderly patients and mice, suggesting that *circFoxo3* may be a marker of cellular senescence (Du et al., 2017). In a doxorubicin-induced cardiomyopathy model, researchers found that silencing *circFoxo3* could alleviate myocardial injury while overexpression of *circFoxo3* aggravated myocardial damage. Further investigation revealed that anti-senescent protein (ID-1), transcription factor (E2F1), and anti-stress proteins (FAK and HIF1a) interacted with *circFoxo3* and were retained in the cytoplasm, leading to increased cellular senescence. Additionally, *circFoxo3* was reported to combine with cyclin CDK2 and cell cycle dependent kinase inhibitor 1 (p21) to form a circFoxo3-p21-CDK2 ternary complex, thus arresting the function of CDK2 and blocking cell cycle progression (Du et al., 2016).

6.3. CircRNA in reproductive aging

Ovarian senescence is an age-related decline of female reproductive capacity, characterized by the gradual reduction in the quantity and quality of oocytes (Qiao et al., 2014; te Velde et al., 1998). Neuroendocrine, genetic and environmental factors are involved in the regulation of ovarian senescence (May-Panloup et al., 2016). ncRNA, especially circRNA, is an emerging potential regulator of gene expression, particularly at the post transcriptional level and is one of the most important members of the epigenetic regulation network (Wei et al., 2017). In 2014, researchers first identified the expression profile of circRNAs in *Drosophila* ovarian tissue (Westholm et al., 2014). Subsequently, the potential effects of circRNAs in preovulatory ovarian follicles of goats were identified (Tao et al., 2017). More recently, Chen et al. (2018b) revealed potential effects in ovary activation and oviposition of honey bees. Notably, Cheng et al. (2017) first characterized global circRNA profiles in human granulosa cells (GCs) and demonstrated the significant difference between young and elderly women. They revealed that elevated expression of circRNA_103827 and circRNA_104816 were closely associated with a declining ovarian reserve and adverse reproductive outcomes. Intriguingly, circRNA_103827 had a predictive ability for pregnancy outcomes after ART cycles, indicating its potential as a new biomarker for IVF outcomes. More recently, by using RNA-seq, our recent study has identified a plenty of novel ovary-derived circRNAs from young and aging women. And *circDDX10-miR-1301-3p/miR-4660-SIRT3* axis might be involved in the regulation of ovarian function during human ovarian senescence although more experiments are needed to confirm this relationship (Cai et al., 2018). Therefore, further deciphering of the molecular mechanisms and biological functions of circRNAs may help improve ART outcomes and uncover the mechanisms of reproductive aging.

6.4. CircRNA in skin aging

Skin aging, also referred to as extrinsic skin aging or photoaging, mainly results from chronic exposure to ultraviolet (UV) radiation (Kohl et al., 2011). A decrease in type I collagen synthesis is an important feature of skin photoaging, but the specific mechanism is not yet fully elucidated (Talwar et al., 1995). Peng et al. (2017) identified 29 significantly differentially expressed circRNAs (12 upregulated and 17 downregulated) in human dermal fibroblasts (HDFs) by RNA-seq. Among these circRNA, the downregulated *circCOL3A1-859267* exhibited the most significant alteration in photoaged HDFs. Functional experiments exhibited that *circCOL3A1-859267* regulated the synthesis of type I collagen but did not affect the expression of type III collagen. Moreover, 44 miRNAs were predicted to bind with *circCOL3A1-859267*, and 5 of them were confirmed to interact with type I collagen. Therefore, *circCOL3A1-859267* is expected to become a new target for the interference of photoaging.

6.5. CircRNA in immunosenescence

During the aging process, the immune system gradually deteriorates, a process known as immunosenescence (Weng et al., 2009). One of the prominent changes of the immune system is the decrease of CD28 expression (Weinberger et al., 2009). In other words, the upregulation of the CD28(-)CD8(+) T subset could be a hallmark of immunosenescence (Parish et al., 2010). By using cross-comparison of circRNA microarrays and stepwise bioinformatics, researchers found that circRNA_100783 may be involved in the loss of CD28 in CD8(+) T cells during aging (Wang et al., 2015). Interaction network analysis of circRNA_100783-miRNA-mRNA revealed that circRNA_100783 might be involved in alternative splicing, production of splice variants, and regulation of phosphoprotein-related signal transduction on CD28-dependent CD8(+) T cell aging. Considering the hypothesis of splicing-related biogenesis of circRNA, the authors proposed that circRNA_100783 might play a role in phosphoprotein-associated functions and may represent a novel biomarker during CD28-related CD8(+) T cell aging and global immunosenescence (Wang et al., 2015).

6.6. CircRNA in visual aging

Aging is associated with decreased neuronal function, which can lead to increased incidence of many neurodegenerative and ocular diseases (Kolesnikov et al., 2010; Shinomori and Werner, 2012). It has recently been proposed that changes in expression of genes in the aging retina could also account for visual senescence (Yang et al., 2015). Hall et al. (2017) demonstrated that the transcriptional landscape of aging photoreceptors was driven largely by transcription factors. Notably, increased expression of genes involved in stress and DNA damage response and decreased expression of genes required for neuronal function were identified; in comparison, circRNA levels strongly increased with age, implying the involvement of other gene expression mechanisms with aging. However, because the samples used in this study were mainly obtained from the nuclei of the neurons, the transcriptional transcripts in the cytoplasm, such as the mitochondrial genes, were less involved, so the enrichment of circRNAs mainly reflects the situation in the nucleus. These findings suggest that circRNAs found in these photoreceptor neurons may be associated with age-associated visual decline and may provide new targets to slow visual senescence.

6.7. CircRNA and other aging processes

In addition to the aforementioned, circRNAs have been also reported to be associated with cellular senescence of primary human fibroblasts and other aging processes like leaf senescence of plants. For example, a large number of senescence related circRNAs (SAC-RNAs) were identified in human diploid WI-38 fibroblasts (Panda et al., 2017). Among them, *circPVT1* was shown to have remarkably reduced levels in senescent fibroblasts and was highly expressed in proliferating fibroblasts. Further analysis demonstrated that *circPVT1* might inhibit the expression of downstream proliferative proteins (such as IGF2BP1, KRAS and HMGA2) by modulating the activity of let-7, thus mediating the cell proliferation phenotype.

The growth and senescence of plant leaves are strictly regulated by tight genetic factors at multiple levels. In a recent study, a total of 168 circRNAs, including 40 novel circRNAs (most of them ecircRNAs), were identified in *Arabidopsis thaliana* leaves (Liu et al., 2017). Notably, six and 35 circRNAs were identified during the G-to-M stage and the M-to-S stage, respectively. Consistent with *C. elegans* (Cortés-López et al., 2018), *Drosophila* (Westholm et al., 2014), and some mammalian animals (Gruner et al., 2016; Lu et al., 2018), the circRNA also displayed an upregulated trend during the lifespan of *Arabidopsis* leaves, irrespective of their host genes, indicating that age-accumulation of circRNAs in postmitotic cells could be a universal phenomenon. Plant hormone signal transduction, porphyrin and chlorophyll metabolism during leaf senescence, which could function as new posttranscriptional regulators in the senescence of *Arabidopsis* leaves according to bioinformatics analysis.

7. Perspectives and conclusions

To date, through the new generation of high-throughput sequencing technology, a large number of circRNAs have been identified in aging cells and tissues. However, the study of circRNA in aging is just beginning. Interestingly, age-dependent neural accumulation of circRNA appears to be a universal phenomenon. The effects of circRNA as a miRNA sponge, protein sponge and transcriptional regulator of the host gene have added new ideas to the complex regulatory network of aging and age-related diseases. We still need to determine if circRNAs have the potential as an aging biomarker, and whether circRNA accumulation play functional roles on the aging nervous system. Perhaps the most intriguing of these ideas is the novel function of the protein translation of circRNA, which opens another door for aging research: do these proteins play a role in the aging process that has been previously unknown? Do these proteins contribute to circRNA accumulation during aging? What is the difference between these proteins and those derived from traditional mRNA translation? Will these small groups of proteins affect the function of traditional proteins? Answering these questions will help us further uncover the mysteries of aging.

Authors' roles

Conception and design (HCC, WPX); literature search, data collection and analysis (HCC, YML, JDN) and interpretation (all authors); drafting the manuscript (HCC); critical revision of the manuscript (all authors).

Conflicts of interest

The authors declare no conflicts of interest.

Funding

This work was supported by National Key Research and Development Program of China (2017YFC1002002).

Acknowledgments

The authors would thank the American Journal Experts (Durham, North Carolina, U.S.A) for language editing.

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