Circular RNAs: Promising Molecular Biomarkers of Human Aging-Related Diseases via Functioning as an miRNA Sponge

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Circular RNAs (circRNAs) are a new class of noncoding single-stranded RNAs that differ from linear microRNAs (miRNAs), since they form covalently closed loop structures without free 3′ poly(A) tails or 5′ caps. circRNAs are the competitive endogenous RNAs (ceRNAs) by binding to miRNA through miRNA response elements (MREs) (i.e., "miRNA sponge"), thereby reducing the quantity of miRNA available to target mRNA, subsequently promoting mRNA stability or protein expression, which involves the initiation and progress of human diseases. Owing to these features of abundance, stability, conservative property, and tissue and stage specificity, widely distributing in the extracellular space and in various bodily fluids, circRNAs can be considered as potential biomarkers for various diseases. Here, we reviewed the promising circRNAs being disease biomarkers, focused on their regulatory function by acting as miRNA sponges, and described their roles in cancer, cardiovascular or neurodegenerative diseases, osteoarthritis, rheumatoid arthritis, diabetes, and other human aging-related diseases, which provide a new direction for pathogenesis, diagnosis, and treatment of human aging-related diseases.

Circular RNAs (circRNAs) are a new class of noncoding single-stranded RNA,[1] characterized by covalently closed loop structures without free 3′ poly(A) tails or 5′ caps. These features differentiate them from linear RNAs,[2] long noncoding RNAs (lncRNAs), and microRNAs (miRNAs).[3] Although circRNAs were first discovered in the 1990s in viruses, viroids, and tetrahymena,[4] little attention has been paid to their function.[4,5] At that time, they were considered abnormal products, resulting from splicing errors.[3,6] In addition, circRNAs are often found in low abundance, and the traditional methods used to study linear RNAs are not applicable. With recent developments in biochemical-enrichment methods, especially high-throughput RNA sequencing and circRNAs microarray, more than 30,000 circRNAs have been discovered.[7] They are widely expressed in yeasts, plants, protists, fruit flies, worms, zebrafish, mice, rats, and humans.[8]

Compared with the levels of their linear isomers, circRNA expression levels can be increased by 10-fold or more,[9] an indication of their potential abundance. Owing to their distinctive structure, they can resist exonuclease activity and are extremely stable.[1] The average lifetime of a 3′ → 5′-linked circRNA is 2∼5 times longer than that of a linear mRNA.[1] In addition, the expression levels of circRNAs are tissue and stage specific, and a number of highly abundant circRNAs have been found to exist in human peripheral blood (PB),[10] indicating that circRNAs can act as biomarkers to screen, diagnose, characterize, and monitor various diseases. circRNAs have many biological functions, including regulating host gene splicing and transcription,[11] acting as miRNA[12] and protein sponges,[8] reacting with proteins,[13] and serving as protein-coding circRNAs.[14] Many studies[15–17] have indicated that circRNAs contain a large number of miRNA binding sites. As competitive endogenous RNAs (ceRNAs), circRNAs can function as an miRNA sponge and competitively combine with the same miRNAs by miRNA response elements (MREs), removing or reducing the inhibition of genes targeted by the miRNAs and regulating the expression of the target genes.[18,19] circRNA not only participates in the proliferation, differentiation, and aging of normal cells but also plays an important role in the pathogenesis of diseases via a large regulatory network of sponge.[20,21] Concerning an important pathway, the novel pathological functions of these circRNA-miRNA-mRNA axes in diseases have been expanded over the last few years,[22] and circRNA-miRNA-mRNA axes cannot only provide a new direction for pathogenesis, diagnosis, and treatment of diseases but also can act as an advanced molecular technology to simulate or manufacture therapeutic agents, which indicates that this regulatory function of circRNAs, by acting as miRNA sponges, should be a focus of research.
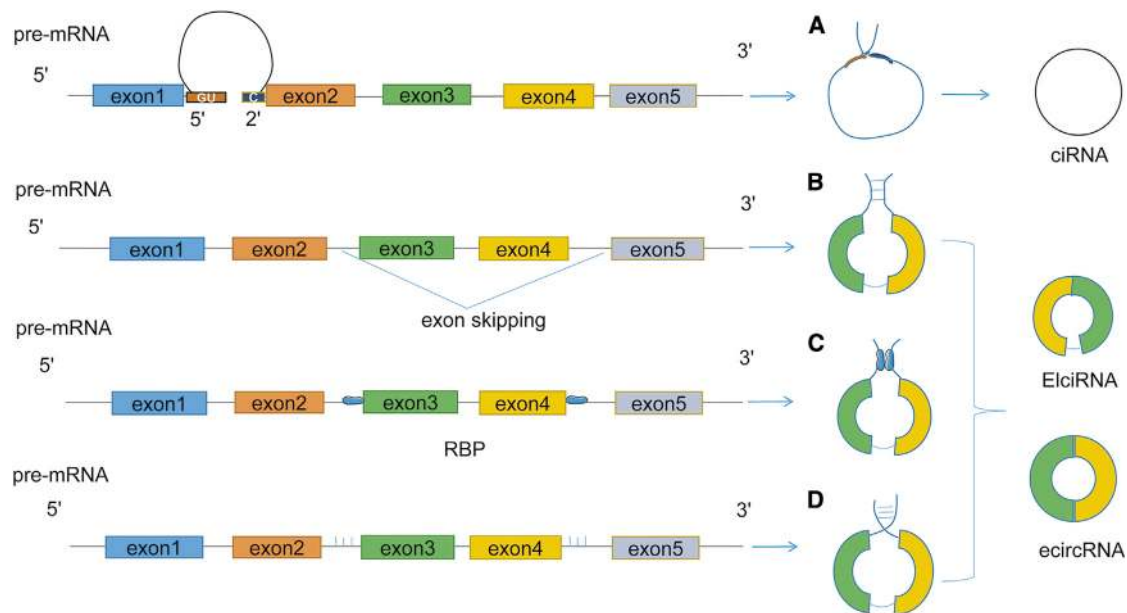
Figure 1. Biogenesis of Circular RNAs (circRNAs) in Eukaryotes

(A) $2'$-$5'$ ciRNAs are generated when abundant GU elements near the $5'$ splice site of the intron and C-rich elements near the branch point escape the debranching enzyme to form an intron lariat. (B–D) ElciRNA and ecircRNA are formed when the downstream splice donor joins the $3'$ acceptor splice site of an upstream exon with the help of exon skipping (B), RNA binding proteins (C), or reverse complementary sequences between the flanking introns (D).

With the gradual aging of the population, the exploration of the biological basis of aging and related molecular mechanisms has become an important topic in modern scientific research.[23] Aging can cause the decay of multiple organ functions, leading to the occurrence and development of various aging-related diseases. These include tumors, heart failure, coronary artery disease (CAD), Alzheimer's disease (AD), osteoporosis, and diabetes. Recently, accumulating evidence has supported the notion that circRNAs are involved in the development of multiple diseases, especially aging-related diseases, such as cancer,[24,25] cardiovascular diseases,[8] neurological disorders,[2] osteoarthritis,[26,27] inflammatory diseases,[28] and diabetes.[29]

Here, we highlight recent advances in our understanding of circRNA biogenesis and expression, focusing on the function of miRNA sponges, which serve as biomarkers in these human diseases.

Biogenesis of circRNAs in Eukaryotes

circRNAs are widespread in the eukaryotic genome. In eukaryotes, RNA polymerase II (RNA Pol II) catalyzes the synthesis of precursor mRNAs (pre-mRNAs). The pre-mRNAs undergo spliceosome-mediated splicing to generate linear mRNAs.[30] Currently, we do not understand the correlation between RNA circularization and alternative splicing, but several sequence features, such as intron/exon length, hyperdebited RNA, and sequence content, affect the formation of circRNAs.[11] It is important to note that the expression levels of circRNAs do not always correlate with those of their linear counterparts, suggesting that the spliceosome can discriminate between linear splicing and RNA circularization, although the mechanism is still unclear.

In terms of their genomic origin, circRNAs can be divided into three classes: circular intronic RNA (ciRNA), exonic circRNA (ecircRNA), and exon-intron circRNA (EIciRNA), all of which possess a covalently closed loop structure. These molecules share multiple features, including stability, abundance, conserved properties, and tissue specificity.[31] ciRNAs, derived from $2' \rightarrow 5'$-linked intronic lariats, are generated by canonical splicing[32] and are mainly found in the nucleus.[33] When pre-mRNAs are spliced, abundant GU elements near the $5'$ splice site of the intron and C-rich elements near the branch point escape the debranching enzyme to form the intron lariat.[8] ecircRNAs, derived only from exons, represent more than 80% of total circRNAs and are located in the cytoplasm. Most circRNAs are generated from exons (protein-coding genes) or from exons and introns through "back-splicing," a noncanonical splicing process.[32,34] In this process, characterized by exon skipping, with the help of RNA binding proteins (RBPs)[24] or reverse complementary sequences between the flanking introns,[31] the downstream splice donor joins the $3'$ acceptor splice site of an upstream exon to form the EIciRNA or circRNA[24,32] (Figure 1). ecircRNAs, containing only exons, are found mainly in the cytoplasm, whereas EIciRNAs, containing exon and intron regions, are located in the nucleus.

As shown in Figure 2, some RBPs, such as muscleblind (MBL),[11] quaking (QKI),[35] fused-in-sarcoma (FUS),[36] RNA binding motif (RBM)20 protein,[37] and double-stranded RNA (dsRNA)-specific adenosine deaminase (ADAR),[32] act as splicing factors and regulate circRNA formation. circMbl is formed by back splicing after MBL binds to MBL binding sites in the flanking introns.[1] On the other
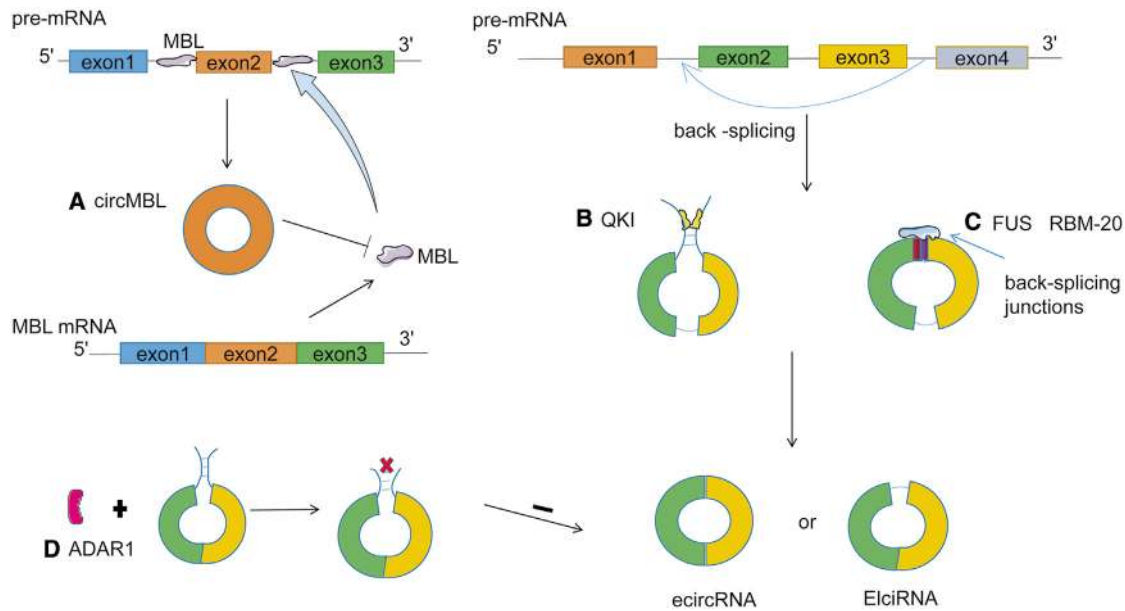
Figure 2. RNA Binding Proteins Regulate the Biogenesis of ecircRNAs and EIciRNAs

(A) circMbl flanking introns have abundant MBL binding sites, MBL promotes the second exon cyclization to form circMbl, and then circMbl and MBL are in dynamic equilibrium. (B) Some introns have QKI binding sites; dimerization through the N-terminal domain of QKI at intron sites can trigger circRNA production. (C) FUS and RBM20 bind to the back-splicing junction to promote circRNA production. (D) ADAR1 negatively regulates circRNA production. ADAR1 converts adenosine to inosine (A-to-I) and breaks RNA-RNA interactions that form during RNA base pairing.

hand, translation of MBL is inhibited by circMbl, which contains the start codon of the main coding sequence,[24] indicating that there is a dynamic balance between MBL and circMbl (Figure 2A). QKI is not only a splicing factor that is essential for circulatory and neural development but also a circRNA regulator that plays an important role in the generation of circRNAs.[1,35] QKI regulates this process depending on the presence of putative QKI binding sites in the flanking introns of circularized exons. These sites are sufficient to induce circRNA biogenesis, suggesting a simple mechanism that regulates the splicing of circRNA through dimerization of flanking introns (Figure 2B).[32] As an RBP, FUS regulates circRNA biogenesis by binding to the introns flanking back-splicing junctions, promoting circRNA production (Figure 2C). Based on this mechanism of splice regulation, studies indicate that FUS participates in the pathogenesis of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia. Similarly, RBM20 also binds to introns flanking back-splicing junctions to regulate the I-band region of the titin transcript, which is known to undergo highly complex alternative splicing and can express approximately 80 circRNAs (Figure 2C).[36,37] The quantity of circRNAs is negatively correlated with the expression levels of the RNA-editing enzyme ADAR1, which disrupts RNA-RNA interactions (Figure 2D). Hence, knockdown of ADAR1 significantly enhances circRNA expression in human HEK293 cells.[38]
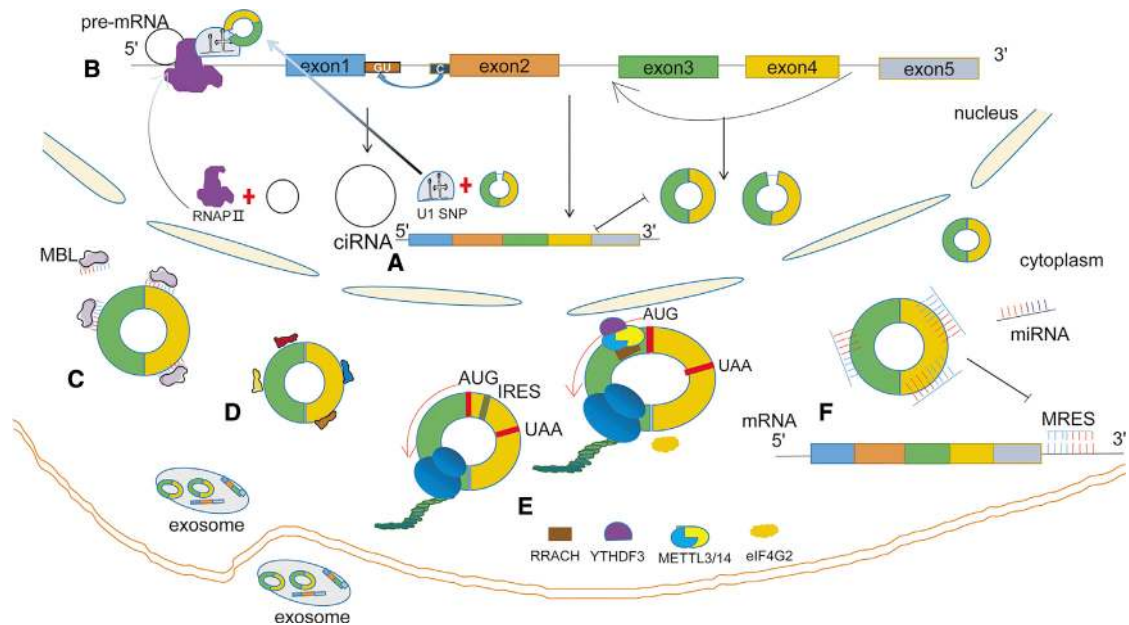
Biological Functions of circRNAs

Although the biological functions of circRNAs remain largely unknown, recent studies have demonstrated that circRNAs participate in several steps of gene expression, for example, competing with their linear cognates to regulate promoter activity,[27] facilitating protein interactions or encoding functional proteins,[13,24] and sponging miRNAs.[12]

In human cells, circRNAs that are located in the nucleus, such as ciRNAs and EIciRNAs,[33] have been found to compete with pre-mRNAs for splicing[14] (Figure 3A). It has been noted that knockdown of ciRNAs derived from the introns of ANKRD52, MCM5, and SIRT7, led to decreased expression of their parent genes without affecting nearby genes, suggesting that these ciRNAs act *in cis*.[39] Another study reported that ciRNAs accumulated at the sites of transcription, interacted with the Pol II elongation complex and regulated transcriptional efficiency.[40] Similarly, EIciRNAs could combine with U1 small nuclear ribonucleoprotein particle (snRNP) via specific RNA-RNA interactions to form EIciRNA-U1-snRNP complexes and recruit RNA Pol II, promoting RNA Pol II activity *in cis* and splicing to enhance host gene transcription[24,41] (Figure 3B). Suppression of EIciRNA-U1-snRNP interactions blocked the transcription-enhancing effects of EIciRNAs.

In addition, circRNAs have been found to sponge or interact with proteins to regulate host gene transcription.[8] For example, circMbl originates in the second exon of the splicing factor MBL. In *Drosophila*, MBL binds to MBL binding sites in the flanking introns to form circMbl.[1] At the same time, MBL can combine with circMbl to inhibit the production of circMbl,[42] indicating that circRNAs not only modulate alternative splicing but can also act as protein sponges

Figure 3. The Functions of Circular RNAs (circRNAs)

(A) circRNAs compete with pre-mRNA linear splicing. (B) circRNAs participate in the process of translation. ciRNAs interact with RNA Pol II (RNAP II), ElciRNAs combine with U1-snRNP via specific RNA-RNA interactions, and ElciRNAs-U1-SNP complexes then recruit RNAP II, promoting RNAP II activity *in cis* and splicing to enhance host gene transcription. (C) circRNAs can act as protein sponges. Some circRNAs, such as circMbl, have protein binding sites. MBL can combine with circMbl to inhibit the production of circMbl. (D) Some circRNAs can interact with proteins directly. (E) Endogenous circRNAs containing an internal ribosome entry site (IRES) and open reading frame (ORF) can also be translated in a 5′ cap-independent way. m6A-modified circRNAs have the ability to translate proteins. circRNAs can be m6A modified via the METTL3/METTL14 complex, and circRNAs containing a m6A modification site can recruit eIF4G2 protein and other translation initiation factors by recruiting the YTHDF3 protein and then initiate the process of translation. (F) circRNAs can act as miRNA sponges. Natural endogenous circRNAs contain numerous miRNA binding sites; by sequence complementary, they can suppress the activity of miRNAs, promoting mRNA translation.

(Figure 3C). Another example is circ-Foxo3 (associated with heart failure), which binds to the age-related proteins ID1 and E2F1, the stress-related protein HIF1a, and FAK in the cytoplasm, inhibiting the entry of these nuclear transcription factors (ID1, E2F1, and HIF1a) into the nucleus, thus inhibiting their function (Figure 3D).[13]

More surprisingly, some circRNAs that contain an internal ribosome entry site (IRES) and open reading frame (ORF) have been demonstrated to have protein-coding potential in a 5′ cap-independent way,[8] thus expanding the eukaryotic proteome. Current evidence indicates that most circRNAs originate from exons and are located in the cytoplasm, which increases the possibility that they may act as protein-coding circRNAs.[43] In gliomas, circ-FBXW7 has been found to encode a novel 21-kD protein called FBXW7-185aa through a cross-knot ORF driven by the ribosome entry site. The expression of FBXW7 and FBXW7-185aa was decreased in gliomas, and this had prognostic significance.[5] In another study, circRNAs, containing an N⁶-methyladenosine (m6A) modification site, were found to recruit eIF4G2 protein and other translation initiation factors by recruiting the YTHDF3 protein and then to initiate protein translation via the METTL3/METTL14 complex (Figure 3E).[14]

More recently, circRNAs have been found to share MREs, allowing them to compete for miRNA binding sites[4,44] and function as miRNA

sponges, sequestering or reducing the quantity of miRNA available to target mRNA[45] and subsequently promoting mRNA stability or protein expression (Figure 3F).[24] CDR1as was the first circRNA seminally reported to act as a miRNA sponge, and it contains over 70 conserved miRNA-7 binding sites.[1,2] Likewise, cSRY is a circRNA that is specifically expressed in murine testes and harbors 16 binding sites for miRNA (miR)-138.[21] Recently, a novel circRNA, termed circHIPK3, originating in exon 2 of the HIPK3 gene, was reported to bind to multiple miRNAs, including miR-124, miR-30a, and miR-558.[46–48]

circRNAs Play an Important Role in Aging-Related Diseases by Acting as miRNA Sponges and Can Be Used as Potential Biomarkers

Based upon their biological functions, circRNAs have been found to be involved in the development of human aging-related diseases, such as cancer,[25] cardiovascular[42] and neurodegenerative[32,49,50] disorders, inflammatory[27] respiratory diseases,[7] and diabetes.[29] In addition, since circRNAs are abundant, stable, and conserved and show organ specificity, they have the potential to serve as diagnostic and prognostic biomarkers.[31,51–53] Here, we focus on the regulatory function of miRNA sponges in human aging-related diseases, as well as the roles of circulating circRNAs as potential diagnostic and prognostic biomarkers.[31,51,52]

circRNAs in Cancer

circRNAs have been well shown to be involved in different aspects of cancer, such as growth, metastasis, stemness, and resistance to therapy.[54]

For example, hsa_circ_0012673,[12] hsa_circ_0000064,[55] circPUM1,[56] hsa_circ_0007385,[57] and circ_0000735[58] were significantly upregulated in lung carcinoma (LC) tissue when compared with normal adjacent tissue, and there was a correlation between the level of expression of these circRNAs and tumor size.[12] *In vitro*, hsa_circ_0012673 was found to promote the proliferation of LC cells by sponging miR-22.[12] Upregulation of circPUM1 has been shown to promote cell proliferation, migration, and invasion in lung adenocarcinoma (LAC)[56] and to inhibit apoptosis through a circPUM1-miR-326-cyclin D1/Bcl-2 axis. Through bioinformatics analysis, hsa_circ_0007385 and circ_0000735 were found to sponge miR-181[57] and miR-1179/miR-1182,[58] respectively, and to promote cell proliferation, migration, and invasion in non-small cell lung cancer (NSCLC). In contrast, downregulated hsa_circ_0043256 in NSCLC[59] acted as a miR-1252 sponge and could directly target a vital negative regulator of canonical Wnt signaling, itchy E3 ubiquitin protein ligase (ITCH). Cinnamaldehyde, the primary chemical component of the Chinese traditional herb *Cinnamomum cassia* and an effective cytotoxic agent against various human cancers, upregulates hsa_circ_0043256 in NSCLC cells, a finding that provides novel insights into lung cancer therapy.[59]

circRNAs have also been shown to serve as biomarkers and therapeutic targets in hepatocellular carcinoma (HCC). For instance, downregulated circMTO1 was found to be positively correlated with a shortened life expectancy,[44] both circMTO1 and hsa_circ_0001445[60] were found in HCC tissues, and circMTO1 led to a reduction in p21 expression levels by sponging miR-9 and promoting HCC cell proliferation and invasion, suggesting that circMTO1 is a potential prognostic biomarker in HCC.[44] On the other hand, it was found that circRNA_100338 regulated metastasis in hepatitis B-related liver cancer by sponging miR-141-3p, and upregulation of circRNA_100338 positively correlated with a low patient survival rate. These findings provide a basis for the possible use of circRNA_100338 as a biomarker to diagnose liver cancer and to understand patient survival rates.[61]

After comparing the circRNA expression profiles of tumoral versus nontumoral tissues, hsa_circ_0001564,[3] hsa_circ_0009910,[62] circ_0000502,[63] and circ-NT5C2[64] were found to be overexpressed in osteosarcoma (OS) tissues and cells. Subsequent bioinformatics analyses showed that hsa_circ_0001564 could sponge miR-29c-3p, circ_000050 could sponge miR-1238,[3] and hsa_circ_0009910 could sponge miR-449a[62] to mediate tumorigenicity. Silencing hsa_circ_0001564,[3] hsa_circ_0009910,[62] and circ_0000502[63] significantly suppressed the proliferative activity of OS cells, induced cell-cycle arrest, and triggered OS cell apoptosis. Based on clinicopathological factors and the prognosis of OS patients, researchers found that circ-NT5C2 could act as a molecular biomarker to diagnose and treat OS.[64]

circRNAs are also aberrantly expressed in gastric adenocarcinoma (GC) tissues. Based on the circRNA expression profile and bioinformatic analysis, circLARP4 might sponge miR-424, which has been shown to inhibit the expression of large tumor suppressor kinase 1 (LATS1) in GC samples and to reduce cell proliferation and invasion, reducing the level of its downstream protein (LATS1).[65] Axes, such as circPSMC3-miR-296-5p-PTEN,[66] circYAP1-miR-367-5p-p27^{Kip1},[67] and circ-SFMBT2-miR-182-5p-CREB1[68] have been shown to regulate GC cell growth.[57]

Abnormal circRNA expression has also been detected in breast cancer (BC) patients, including both upregulated circRNAs associated with the Hippo and Wnt signaling pathways (such as hsa_circ_103110, hsa_circ_104689, and hsa_circ_104821)[64] and downregulated circRNAs associated with the RAP1 and Ras signaling pathways (such as hsa_circ_006054, hsa_circ_100219, and hsa_circ_406697).[69] Putative interactions between circRNAs and miRNAs[69] during the initiation and progression of BC have also been predicted. For example, circRNA-000911, which was downregulated in BC tissues and cell lines, could regulate Notch1 expression by sponging miR-449a, hence regulating nuclear factor κB (NF-κB) signaling.[70] circRNA-miRNA axes involving ceRNAs, such as TCDD-hsa_circ_0001098-miR-3942-BARD1,[71] hsa_circ_001783-miR-200c-3p,[72] hsa_circ_0052112-miR-125a-5p,[73] and circ_0005230-miR-618-CBX8, have been found in BC.[74] circTADA2A-6E-miR-203a-3p-SOCS3[75] and circAGFG1-miR-195-5p-CCNE1[76] have been found in triple-negative breast cancer (TNBC).

Interactions between circRNAs and miRNAs have also been demonstrated in other malignant tumors. For example, the circRNA myosin light chain kinase (MYLK), upregulated in bladder cancer (Bc) tissues and cell lines,[77] could act as a miR-29a sponge to increase the expression of VEGFA and activate the vascular endothelial growth factor A (VEGFA)/vascular endothelial growth factor 2 (VEGFR2) signaling pathway and the downstream Ras/extracellular signal-regulated kinase (ERK) signaling pathway, accelerating the progression of Bc. By sponging miR-30c, circPRMT5 could regulate SNAIL1/E-cadherin and induce epithelial-mesenchymal transition, which was positively associated with an advanced clinical stage and worse survival of patients with urothelial carcinoma of the bladder (UCB).[78] By comparing the expression levels in colorectal cancer (CRC) tissues with those in adjacent nontumoral tissues or many different types of normal cells, hsa_circ_0007534[79] was found to be upregulated, and circMTO1 was found to be downregulated in CRC.[80] In addition, their abnormal levels of expression were consistent with the tumor stage. hsa_circ_0007534 and circMTO1 are promising as prognostic biomarkers and therapeutic targets in CRC. In addition, the expression of hsa_circ_0001649[6] has been shown to be reduced in cholangiocarcinoma (CCA), and the expression of circRNA_100290[81] has been found to be upregulated in oral squamous cell carcinoma (OSCC).

circRNA expression profiles have also been correlated with gliocytoma. For example, it was shown that circ-TTBK2 sponged miR-217 in a sequence-specific manner, evidenced by the fact that

upregulated circ-TTBK2 decreased miR-217 expression, and there was reciprocal negative feedback between them in an argonaute 2-dependent manner. In addition, reintroduction of miR-217 significantly reversed the circ-TTBK2-mediated promotion of glioma progression. Particularly convincing was the demonstration that circ-TTBK2 knockdown combined with miR-217 overexpression led to tumor regression *in vivo*.[82]

The roles of these previously mentioned circRNAs and circRNAs/ miRNAs in cancer regulation are listed in Table 1.

circRNAs in Cardiovascular Diseases

A comprehensive understanding of the role of circRNAs in the pathogenesis of cardiovascular diseases may permit the identification of biomarkers and therapeutic targets for CAD,[83] heart failure,[84] and myocardial infarction.[85]

An in-depth investigation of the mechanisms has suggested that the occurrence and development of CAD might be related to the joint regulation of circRNAs and miRNAs.[86] It has been shown that circNCX1, which acts as a sponge of miR-133a-3p, is significantly increased in reactive oxygen species (ROS)-producing cardiomyocytes and promotes cardiomyocyte apoptosis by targeting CDIP1.[87] In terms of myocardial fibrosis (MF), secondary to myocardial ischemia and hypoxia, caused by moderate/severe coronary atherosclerotic stenosis, circRNA_010567[88] and circRNA_000203[31] were shown to be significantly upregulated in a model of MF in mice. Through bioinformatics analysis, it was found that circRNA_010567 could sponge miR-141 at more than one binding site, and the 3′ UTR of transforming growth factor β1 (TGF-β1) was highly correlated with miR-141.[88] This was verified by the fact that overexpression of circRNA_010567 promoted the expression of TGF-β1 and promoted the expression of the fibrosis-related proteins collagen type I (Col I), Col III, and α-smooth muscle actin (α-SMA).[88] circRNA_000203 was found to bind to miR-26b-5p, which bound to the 3′ UTRs of collagen type I α 2 chain (Col1a2) and connective tissue growth factor (CTGF), and this was verified by the fact that overexpression of circRNA_000203 directly promoted the expression of Col1a2 and CTGF.[31] However, the correlation between circRNA_010567 and circRNA_000203 in the pathological process of MF is still unclear.

Cardiac hypertrophy (CH) is associated with a significantly increased risk of heart failure (HF), one of the leading causes of death in the world.[84] A recent study showed that the expression of mm9-circ-012559, named heart-related circRNA (HRCR), was significantly reduced in an isoproterenol- or aortic arch constriction-induced model of CH in mice, and bioinformatics analysis revealed that HRCR contains six binding sites for miR-223, which targets the 3′ UTR of ARC (apoptosis repressor with CARD domain) to promote CH;[84] these findings indicate that mm9-circ-012559 could be a therapeutic target in CH.

Acute myocardial infarction (AMI) has a 30-day mortality rate approaching 10% in most European and North American countries.[85]

circRNA_081881 was found to be significantly downregulated in the plasma of AMI patients, and western blot analysis showed that the expression of peroxisome proliferator-activated receptor gamma (PPARγ) correlated with circRNA_081881 levels. Additional experiments confirmed that circRNA_081881 sponged miR-548 to regulate the expression of PPARγ in AMI, indicating that it was a potential target for the diagnosis and treatment of AMI.[89]

The roles of these above-mentioned circRNAs and circRNAs/miR-NAs in cardiovascular disease regulation are listed in Table 2.

circRNAs in Neurodegenerative Diseases

Certain circRNAs have been found to be specifically expressed in the mammalian central nervous system,[38] suggesting that they play tissue-specific physiological and pathological roles and may also serve as potential diagnostic biomarkers or therapeutic targets in diseases that affect this organ system.

For example, in rats with sciatic nerve injury (SNI), the miR-34 family connected circRNAs to LC3-II/p62, as evidenced by the fact that circRNA_2837 sponged miR-34 family members and induced autophagy, which in turn, protected neurons from damage.[90] In HT22 cells subjected to hypoxia/reoxidation, mmu-circRNA-015947 was upregulated and sponged certain miRNAs associated with neural damage (such as mmu-miR-188-3p, mmu-miR-329-5p, mmu-miR-3057-3p, mmu-miR-5098, and mmu-miR-683), regulating their target gene expression. Based on Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, mmu-circRNA-015947 has been shown to be associated with pathological processes, such as apoptosis, metabolism, and the immune response during cerebral ischemia-reperfusion.[91]

circRNAs tend to accumulate during the normal brain aging process and thus, may correlate with susceptibility to aging-related neurodegenerative diseases, such as AD. The role of circRNAs in AD has gradually been recognized.[92] For example, evolutionarily conserved miRNA-7 was abundant in the human brain, and its levels correlated with ciRS-7 (also known as CDR1as), a circRNA specific for miRNA-7 and containing multiple tandem anti-miRNA-7 sequences; in addition, ciRS-7 acts as an endogenous anticomplementary miRNA "sponge" to adsorb and quench normal miRNA-7 activity.[93] Downregulation of ciRS-7 and ciRS-7 "sponging activity" increased miRNA-7 levels in AD-affected brain cells, ultimately contributing to the downregulation of miRNA-7-sensitive mRNA targets.[94] Deficits in other circRNA-mediated "miRNA-sponging systems" and the consequent upregulation of certain endogenous miRNAs may explain the generalized and progressive downregulation of gene expression that is characteristically seen in sporadic AD.[95–97]

Parkinson's disease (PD) is another aging-related neurodegenerative disorder and mainly affects substantia nigra neurons in the brain, causing a common type of dyskinesia.[98] It has been reported that miR-7 can inhibit the expression of α-synuclein, a crucial constituent of Lewy bodies in the PD brain, and protect against oxidative stress

Table 1. circRNAs Involved in Cancer

Cancers	circRNAs	Method	+/−	Axis	Effects	Samples	Ref.
LC	hsa_circ_0012673	circRNA-array	+	hsa_circ_0012673-miR-22-ErbB3	positive correlates to tumor size; promotes LAC cell proliferation	human LC tissue	12
LC	hsa_circ_0000064	N/A	+		promotes cell proliferation and metastasis	human LC tissue and cell lines	55
LAC	circPUM1	N/A	+	circPUM1-miR-326-cyclin D1 and Bcl-2	promotes LAC cell proliferation, migration, and invasion and inhibits apoptosis	human LAC tissue and cell lines	56
NSCLC	hsa_circ_0007385	circRNA-array	+	hsa_circ_0007385-miR-181	promotes cell proliferation, migration, and invasion	human NSCLC tissues	57
NSCLC	circ_0000735	circRNA-array	+	circ_0000735-miR-1179/miR-1182	associates with more advanced TNM stages; promotes cell proliferation, migration, and invasion	human NSCLC tissues	58
NSCLC	hsa_circ_0043256	circRNA-array	−	hsa_circ_0043256-miR-1252-ITCH	promotes cell proliferation and inhibits apoptosis and further directs Wnt/β-catenin pathway	human NSCLC cell lines	59
HCC	circMTO1	circRNA-array	−	circMTO1-miR-9-P21	parallel with the shortening of life; promotes HCC cell proliferation and invasion	human HCC tissues	44
HCC	hsa_circ_0001445	N/A	−		promotes HCC cells proliferation, migration, and invasion and inhibits apoptosis	human HCC tissues and cell lines	60
HCC	circRNA-100338	circRNA-array	+	circRNA-100338-miR-141-3p	positively correlates with the cumulative survival rate and cancer metastasis	human HCC tissues	61
OS	hsa_circ_0001564	circRNA-array	+	hsa_circ_0001564-miR-29c-3p	silencing of hsa_circ_0001564 significantly suppresses proliferation and promotes apoptosis in HOS and MG-63 cells	human OS tissue	3
OS	hsa_circ_0009910	N/A	+	hsa_circ_0009910-miR-449a-IL6R	knockdown of circ_0009910 inhibits OS cell proliferation; induces cell-cycle arrest and apoptosis	human OS tissue and cell lines	62
OS	circ_0000502	circRNA-array	+	circ_0000502-miR-1238	relates to clinical severity; facilitates OS cell proliferation, migration, and invasion; and inhibits apoptosis	human OS tissue and cell lines	63
OS	circ-NT5C2	N/A	+		may be a molecular biomarker to diagnose and treat OS	human OS tissues	64
GC	RNA_LARP4	circRNA-array	−	RNA_LARP4-miR-424-5p-LATS1	associates with pathological stage and unfavorable prognosis of GC patients; promotes GC cell proliferation and invasion	GC tissues	65
GC	circPSMC3	circRNA-array	−	circPSMC3-miR-296-5p-PTEN	correlates with higher TNM stage and shorter overall survival; overexpression of circPSMC3 suppresses the proliferation and invasion and inhibits the growth and metastasis of GC cells	human GC tissues, plasma, and cell lines	66
GC	circYAP1	N/A	−	circYAP1-miR-367-5p-p27^{Kip1}	positively correlates survival of GC patients; overexpressed circYAP1 inhibits the proliferation and invasion of GC cells	human GC tissues	67
GC	circ-SFMBT2	circRNA-array	+	circ-SFMBT2-miR-182-5p-CREB1	associates with higher tumor stages of GC; promotes the proliferation of GC cells	human GC tissues and cell lines	68
BC	hsa_circ_103110, hsa_circ_104689, hsa_circ_104821, hsa_circ_006054,	circRNA-array			hsa_circ_103110, hsa_circ_104689, and hsa_circ_104821(+) associate	BC tissues	69

(Continued on next page)

Table 1. Continued

Cancers	circRNAs	Method	+/−	Axis	Effects	Samples	Ref.
	hsa_circ_100219, and hsa_circ_406697				with Hippo and the Wnt signaling pathway, and hsa_circ_006054, hsa_circ_100219, and hsa_circ_406697(−) associate with RAP1 and the Ras signaling pathway		
BC	circRNA-000911	circRNA-array	−	circRNA-000911-miR-449a-Notch1	overexpression of circRNA-000911 suppresses cell proliferation, migration, and invasion and promotes the apoptosis of BC cells	human BC tissues and cell lines	[70]
BC	hsa_circ_0001098	circRNA-array	+	hsa_circ_0001098-miR-3942-BARD1	inhibits cell proliferation and promotes cell apoptosis	human BC (treated with TCDD) tissues and cells	[71]
BC	hsa_circ_001783	N/A	+	hsa_circ_001783-miR-200c-3p	correlates with poor clinical outcomes in BC patients; promotes the progress of BC	BC tissues and cell lines	[72]
BC	hsa_circ_0052112	circRNA-array	+	hsa_circ_0052112-miR-125a-5p	promotes cell migration and invasion in BC	MDA-MB-231 cells	[73]
BC	circ_0005230	circRNA-array	+	circ_0005230-miR-618-CBX8	relates to adverse phenotypes in the patients with BC; as a prognostic predictor in BC patients; promotes BC cell growth and migratory and invasive capacities	BC tissues and cell lines	[74]
TNBC	circTADA2As	circRNA-array	−	circTADA2A-6E-miR-203a-3p-SOCS3	may be prognostic biomarkers for BC	TNBC tissues	[75]
TNBC	circAGFG1	HT RNA-seq	+	circAGFG1-miR-195-5p-CCNE1	associates with the progression and poor prognosis; promotes TNBC cell proliferation, migration, and invasion and modulates cell cycle and apoptosis; facilitates tumorigenesis, angiogenesis, and metastasis of TNBC cells *in vivo*	human TNBC tissues and cell lines	[76]
Bc	circRNA-MYLK	circRNA-array	+	circRNA-MYLK-miR-29a-VEGFA	relates to the progression of stage and grade of BC; accelerates cell proliferation, migration, and tube formation of HUVEC; rearranges cytoskeleton; promotes EMT through activating VEGFA/VEGFR2 signaling pathway	Bc tissues and cell lines	[77]
UCB	circPRMT5	circRNA-array	+	circPRMT5-miR-30c-SNAIL1/E-cadherin	associates with advanced clinical stage and worse survival; correlates with tumor metastasis	UCB tissues, exosome purification, serum, and urine	[78]
CRC	hsa_circ_0007534	circRNA-array	+		correlates with tumor stage and lymph node metastasis; promotes cell proliferation; and prevents apoptosis	CRC tissues and cell lines	[79]
CRC	circMTO1	N/A	−		correlates with advanced TNM stage, lymph node metastasis, and poor overall survival; promotes cell proliferation and invasion	CRC tissues and cell lines	[80]
CCA	hsa_circ_0001649	N/A	−		associates with tumors size and differentiation grade; promotes cell proliferation, migration, and invasion; inhibits apoptosis	CCA tissues and cell lines	[6]
OSCC	circRNA_100290	circRNA-array	+	circRNA_10 0290-miR-29 family-CDK6	promotes cell proliferation	OSCC tissues and cell lines	[81]

(Continued on next page)

Table 1. Continued

Cancers	circRNAs	Method	+/−	Axis	Effects	Samples	Ref.
Gliocytoma	circ-TTBK2	N/A	+	circ-TTBK2-miR-217-HNF1β/Derlin-1	promotes cell proliferation, migration, and invasion, whereas inhibits apoptosis	Gliocytoma tissues and cell lines	82

LC, lung carcinoma; LAC, lung adenocarcinoma; NSCLC, non-small cell lung cancer; HCC, hepatocellular carcinoma; OS, osteosarcoma; GC, gastric cancer; EMT, epithelial-mesenchymal transition; BC, breast cancer; TNBC, triple-negative breast cancer; Bc, bladder cancer; UCB, urothelial carcinoma of the bladder; CRC, colorectal cancer; CCA, cholangiocarcinoma; OSCC, oral squamous cell carcinoma; HT RNA-seq, high-throughput RNA sequencing; circRNA-array, circRNA microarray; N/A, not available; +, upregulate; −, downregulate; Ref., references; HUVEC, human umbilical vein endothelial cell; CDK6, cyclin-dependent kinase 6; HNF1β, hepatocyte nuclear factor 1beta.

and the neuronal death induced by 1-methyl-4-phenylpyridinium (MPP$^+$) by targeting the NF-κB signaling pathway.[99–101] In addition, another report indicated that circDLGAP4 was decreased in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD mouse model and MPP$^+$-induced PD cell models. Bioinformatics studies suggested that circDLGAP4 acted as a miR-134-5p sponge to regulate CREB signaling and influence the expression of CREB target genes, including brain-derived neurotrophic factor (BDNF), Bcl-2, and peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), in SH-SY5Y and MN9D cells.[102]

circRNAs in Osteoarthritis

Osteoarthritis (OA) is a common joint disease characterized by degeneration of articular cartilage.[24] In the interleukin (IL)-1β-induced model of arthritis, 255 circRNAs were found to be abnormally expressed, 119 of which were significantly upregulated and 136 of which were downregulated. Gene Ontology (GO) and KEGG pathway analyses revealed that the target genes of these circRNAs were involved in cell metabolism, regulation, and apoptosis. Investigators identified and validated two differentially expressed circRNAs (mmu-circRNA-30365 and mmu-circRNA-36866) that were predicted to function as miRNA sponges. This suggests that circRNAs represent novel molecular targets in OA.[24] In another IL-1β-induced model of arthritis, researchers identified a circRNA (circRNA_Atp9b) that was significantly upregulated in chondrocytes and regulated the expression of downstream genes by targeting miR-138-5p, which modulated extracellular matrix (ECM) metabolism and inflammation. This further confirmed that circRNAs can be used as therapeutic targets in OA.[27] One study investigated the expression of circRNAs in different regions affected by OA and identified a mechanical stress-related circRNA (circRNA-MSR) that participated in the degradation of chondrocytes by targeting miR-875 to regulate tumor necrosis factor α (TNF-α) expression.[103] Additional studies of the process of cartilage degradation have determined that increased levels of IL-1 and TNF-α upregulate ECM-associated circRNA (circRNA-CER). circRNA-CER competed with miR-136 for MMP-13 RNA.[104]

circRNAs in Diabetes

Progress has been made in exploring the potential role of noncoding RNAs (ncRNAs) in the regulation of gene networks involved in metabolic diseases. Recently, it was demonstrated that circHIPK3 mediated retinal vascular dysfunction in diabetes mellitus (DM) by sponging miR-30a to increase the expression of its target genes.[105]

Finally, expression of circ_010567 was increased in the myocardium and cardiac fibroblasts of diabetic mice and acting through a mechanism involving miR-141 sponging, led to upregulation of TGF-β1 expression.[88]

circRNAs in Other Diseases

In the lipopolysaccharide (LPS)-induced model of respiratory distress syndrome, five differentially expressed circRNAs were selected and validated based on high-throughput detection of circRNAs: mmu-circRNA_19423, rno_circRNA_010489, rno_circRNA_011426, and mmu-circRNA_30664 were significantly upregulated, whereas rno_-circRNA_005564 was significantly downregulated.[106] This result indicates that these circRNAs could potentially be used to diagnose respiratory distress syndrome.

The roles of the previously mentioned circRNAs and circRNAs/miRNAs in the regulation of other diseases are listed in Table 3.

Circulating circRNAs Act as Potential Biomarkers for Diseases

Owing to their covalently closed loop structures, circRNAs can resist degradation by RNases, making them highly stable in plasma, serum, or other biofluids.[107] With the development of high-throughput RNA sequencing, several hundred to thousands circRNAs with tissue- and stage-specific characteristics have been identified, and these circRNAs are found in higher levels in PB than intracellular fractions of relevant solid tissues;[10] as such, the above-mentioned circulating circRNAs have potential to act as biomarkers to screen, diagnose, characterize, and monitor diseases, such as cancers, cardiovascular diseases, inflammatory respiratory diseases, diabetes, and others.

circRNA expression profiles between GC patients and healthy individuals were compared, and circ-KIAA1244 was found to be expressed at lower levels in GC plasma than in plasma from healthy individuals and was negatively correlated with tumor node metastasis (TNM) stage, lymphatic metastasis, and a shorter overall survival time of GC patients, which indicated that circ-KIAA1244 could serve as a novel circulating biomarker for detecting GC.[108] In addition, hsa_circ_0000520[109] and circ_0009910[110] were anomalously expressed in GC plasma and had the potential to be used as prognostic biomarkers. However, the correlation among circ-KIAA1244, hsa_-circ_0000520, and circ_0009910 still needs to be researched. Another study indicated the potential of hsa_circ_0005962 and

Table 2. circRNAs Involved in Cardiovascular Diseases

Diseases	circRNAs	Method	+/−	Axis	Effects	Samples	Ref.
IMI	circNCX1	Sanger sequencing	+	circNCX1-miR-133a-3p-CDIP1	promotes cardiomyocyte apoptosis	fetal cardiomyocyte-derived H9c2 cell	[87]
MF	circRNA_010567	circRNA-array	+	circRNA_010567-miR-141-TGF-β1	promotes Col I, Col III, and α-SMA expression	diabetic mice myocardium and CFs treated with Ang II	[88]
MF	circRNA_000203	circRNA-array	+	circRNA_000203-miR-26b-5p-Col1a2 and CTGF	promotes the expression of Col1a2 and CTGF and promotes myocardial fibrosis	myocardium of the db/db mice	[31]
CH and HF	HRCR/(mm9-circ-012559)	N/A	−	HRCR-miR-223-ARC	overexpression of HRCR can decrease cardiomyocyte size, W_H/W_B, cardiac stress gene expression, and interstitial fibrosis	mouse heart	[84]
AMI	circRNA_081881	circRNA-array	−	circRNA_081881-miR-548-PPARγ	as a potential target for the diagnosis and treatment of AMI	plasma of AMI patients	[89]

IMI, ischemic myocardial injury; MF, myocardial fibrosis; CFs, mouse cardiac fibroblasts; CH and HF, cardiac hypertrophy and heart failure; W_H/W_B, heart weight to body weight ratio; AMI, acute myocardial infarction; circRNA-array, circRNA microarray; N/A, not available; +, upregulate; −, downregulate.

hsa_circ_0086414 as circulating circRNAs to diagnose LAC.[111] Circulating exosomal hsa_circ-0004771 was significantly upregulated in CRC patients and served as a novel potential diagnostic biomarker of CRC.[112] Based on receiver operating characteristic (ROC) curve analysis and its correlation with clinical characteristics, such as histological grade, TNM stage, and distant metastasis, hsa_circ_0001785, as a circulating circRNA in the PB of BC patients, was selected as the best diagnostic marker for BC.[113] In UCB, circPRMT5 was positively associated with advanced clinical stage and poor survival of patients.[78]

Early diagnosis of congenital heart disease (CHD) could improve disease prognosis in childhood and reduce mortality.[114] One study indicated that three circRNAs (hsa_circRNA_004183, hsa_circRNA_079265, and hsa_circRNA_105039) were significantly downregulated in the plasma of children with CHD, suggesting that these circRNAs may serve as novel plasma biomarkers for the diagnosis of CHD in children.[114] Based on the differential expression profiles in PB determined by RNA microarray and ROC curve analysis, hsa_circ_0124644 has been postulated as a diagnostic biomarker for CAD[83]. In the PB of patients with AMI, the expression of myocardial infarction-mediated circRNA (MI-CRA) was downregulated. Univariate and multivariate logistic regression analyses showed that MICRA was a powerful predictor of left ventricular dysfunction—the lower the MICRA levels were, the higher the risk of left ventricular dysfunction.[115] Investigators determined that MICRA expression levels after AMI were associated with three separate risk stratification levels (3EF), further demonstrating that MICRA could be used as a circulating biomarker for risk prediction after AMI.[85]

Rheumatoid arthritis (RA) is a chronic synovitis of unknown etiology that can lead to joint deformities and loss of function.[23,116] By comparing circRNA expression in peripheral blood mononuclear cells (PBMCs) of RA patients with healthy volunteers, the following circRNAs were identified: hsa_circRNA_104194, hsa_circRNA_104593,

hsa_circRNA_103334, hsa_circRNA_101407, and hsa_circRNA_102594, which suggested that these circRNAs could potentially be used as circulating circRNAs to diagnose and treat RA.[28] Other studies identified and validated five differentially expressed circRNAs (circRNAs 092516, 003524, 103047, 104871, and 101873) with high area under the curve (AUC) values in PBMCs of RA patients, suggesting that these circRNAs could serve as diagnostic biomarkers for RA.[103] In addition, hsa_circ_0044235 was decreased significantly in the PB of RA patients, and ROC curve analysis suggested that hsa_circ_0044235 had significant value in the diagnosis of RA.[117]

hsa_circ_0054633 was abnormally expressed in the PB of diabetic patients, and two cohort tests determined that hsa_circ_0054633 showed a good AUC. At the same time, GO analysis showed that hsa_circ_0054633 participated not only in biological processes but also in molecular metabolic processes. The above results suggested that hsa_circ_0054633 could be used as a diagnostic marker for prediabetes and type 2 diabetes.[29]

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (Mtb) and a leading cause of global morbidity and mortality.[45] Identification of early diagnostic biomarkers for TB would benefit the control of infection. By analyzing the expression profiles of circRNAs in PBMCs of TB patients, researchers found that hsa_circRNA_001937 was significantly upregulated in pulmonary tuberculosis compared with other lung diseases, such as pneumonia, lung cancer, or chronic obstructive pulmonary disease (COPD). Furthermore, with recovery from TB, the expression of hsa_circRNA_001937 was reduced. Likewise, analysis of the ROC curve of hsa_circRNA_001937 suggested that its AUC was high, indicating that this circRNA could be used as a circulating biomarker for the diagnosis and treatment of TB.[45]

Based on the special characteristics of circRNAs and their roles, detailed in the above discussions, in various aging-related diseases, circulating circRNA-based diagnostic and prognostic tools for

Table 3. circRNAs Involved in Other Diseases

Diseases	circRNAs	Method	+/−	Axis	Effects	Samples	Ref.
SNI	circRNA.2837	HT RNA-seq	−	circRNA.2837-miR-34 family-LC3-II/p62	protects neurons against injury by inducing autophagy	ISN of rats	90
NI	mmu-circRNA-015947	circRNA-array	+	mmu-circRNA-015947-mmu-miR-(188-3p, 329-5p, 3057-3p, 5098, 683)	may be involved in the process of IRI	HT22 cells with OGD/R	91
AD	ciRS-7	N/A	−	ciRS-7-miRNA-7-UBE2A	regulates the downregulation of UBE2A expression	human AD brain tissues	93,94
PD	circDLGAP4	N/A	−	circDLGAP4-miR-134-5p-CREB signaling	inhibits viability, induces apoptosis, increases mitochondrial damage, attenuates autophagy, and thereby increases the neurotoxic effects	MPTP-induced PD mouse model and MPP⁺-induced PD cell models	102
OA	mmu-circRNA-30365; mmu-circRNA-36866	HT RNA-seq			new molecular targets for treatment of OA	IL-1β-induced MACs	24
OA	circRNA_Atp9b	N/A	+	circRNA_Atp9b-miR-138-5p-type II collagen/MMP13/IL-6 and COX-2	knockdown of circRNA_Atp9b increases the synthesis of type II collagen, reduces the expression of MMP13, IL-6, and COX-2	IL-1β-induced MACs	27
OA	circRNA-MSR	circRNA-array	+	circRNA-MSR-miR-875-TNF-α	silencing of circRNA-MSR will downregulated TNF-α and increase ECM formation	knee joint tissues of patients	103
OA	circRNA-CER	circRNA-array	+	circRNA-CER-miR-136-MMP13	silencing of circRNA-CER will downregulate MMP13 expression and increase ECM formation	knee joint tissues of OA patients	104
DM	circHIPK3	N/A	+	circHIPK3-miR-30a-VEGFC/Wnt2/FZD4	silencing circHIPK3 can alleviate retinal vascular dysfunction	human and mouse retinal endothelial cells	105

SNI, sciatic nerve injury; ISN, injured sciatic nerve; NI, neuron injury; IRI, cerebral ischemia-reperfusion injury; OGD/R, oxygen-glucose deprivation/reoxygenation; AD, Alzheimer's disease; PD, Parkinson's disease; OA, osteoarthritis; MACs, mouse articular chondrocytes; ECM, extracellular matrix; RA, rheumatoid arthritis; T2DM, type 2 diabetes mellitus; DM, diabetes mellitus; HT RNA-seq, high-throughput RNA sequencing; circRNA-array: circRNA microarray; N/A, not available; +, upregulate; −, downregulate; COX-2, cyclooxygenase 2.

diseases could become a reality. The roles of the previously mentioned circulating circRNAs as potential biomarkers for aging-related diseases are listed in Table 4.

Conclusions

Elucidation of the function of circRNAs allows us to understand their role in health and disease. Due to their structural stability and presence in exosomes, circRNAs can act in an autocrine, a paracrine, and possibly even an endocrine manner. In addition, the fact that circRNAs are widely distributed not only within cells but also in the extracellular space and in various bodily fluids makes them ideal biomarker candidates for various human diseases. As with any emerging field, caution is advised when interpreting the new findings. To date, studies are merely descriptive in nature. More in-depth analysis of the function and regulation of identified circRNAs is needed to understand their mechanism of action. These RNA molecules hold great promise as disease biomarkers because of their high resistance to exonuclease activity and because they might even be more highly expressed than their linear counterparts. Their putative role as miRNA sponges makes them interesting targets for future investigations. In the future, circRNA-based therapies could become a reality. However, the concept of circRNA-based therapies depends on their stability, differential expression in distinct organs, and specificity

for a certain disease. As is the case for miRNAs, the likelihood of triggering significant and undesired off-target effects can greatly limit the use of circRNAs as therapeutics. Hence, the more specific circRNAs are to a specific organ or disease, the less likely they will be to have off-target effects. Elucidation of the role of specific circRNAs is a prerequisite for targeted RNA-based therapies for specific diseases. Alternatively, tissue/cell specificity might be enhanced by coupling ncRNAs to tissue-specific antibodies and/or peptides, thereby reducing off-target effects.

Limitations

circRNAs are being increasingly studied in the RNA field. However, there are still some issues that need to be resolved. How are circRNAs degraded? What other biological functions do circRNAs have in health and disease? How can we standardize the naming of circRNAs? Finally, in a given disease, some circRNAs are upregulated, whereas others are simultaneously downregulated. What are the regulatory relationships between upregulated and downregulated circRNAs? These issues need to be explored in more detail.

AUTHOR CONTRIBUTIONS

Conceptualization, L.S.; Writing – Original Draft preparation, L.S. and S.R.; Writing – Review & Editing, L.S.; Funding Acquisition,

Table 4. Circulating circRNA Acted as a Potential Biomarker for Diseases

Diseases	circRNAs	Method	+ / −	Samples	Ref.
GC	circ-KIAA1244	circRNA-array	−	plasma of GC patients	[108]
GC	hsa_circ_0000520	circRNA-array	−	plasma of GC patients	[109]
GC	circ_0009910	N/A	+	plasma of GC patients	[110]
LAC	hsa_circ_0005962; hsa_circ_0086414	N/A		plasma of LAC patients	[111]
CRC	hsa_circ-0004771	N/A	+	exosomes from serum of CRC patients	[112]
BC	hsa_circ_0001785	circRNA-array		PB of BC patients	[113]
UCB	circPRMT5	circRNA-array	+	UCB tissues, exosome purification, serum, and urine	[78]
CHD	hsa_circRNA_004183, hsa_circRNA_079265, and hsa_circRNA_105039	circRNA-array	−	plasma of children with CHD	[114]
CAD	hsa_circ_0124644	circRNA-array	+	PB of CAD patients	[83]
AMI	MICRA	N/A	−	PB of AMI patients	[85,115]
RA	hsa_circRNA (104194, 104593, 103334, 101407, and 102594)	circRNA-array		PBMC of RA patients	[28]
RA	circRNAs (092516, 003524, 103047, 104871, and 101873)	circRNA-array	+	PBMC of RA patients	[103]
RA	hsa_circ_0044235	circRNA-array	−	PB of RA patients	[117]
T2DM	hsa_circ_0054633	circRNA-array		PB of T2DM patients	[29]
TB	hsa_circRNA_001937	circRNA-array	+	PBMC of TB patients	[45]

GC, gastric adenocarcinoma; LAC, lung adenocarcinoma; CRC, colorectal cancer; BC, breast cancer; UCB, urothelial carcinoma of the bladder; CHD, congenital heart disease; CAD, coronary artery disease; AMI, acute myocardial infarction; RA, rheumatoid arthritis; T2DM, type 2 diabetes mellitus; TB, tuberculosis; PB, peripheral blood; PBMC, peripheral blood mononuclear cell; circRNA-array, circRNA microarray; N/A, not available; +, upregulate; −, downregulate.

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