Review

# The role of circular RNAs in brain and stroke

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## 1. Abstract

Circular RNAs are single-stranded RNAs which are closed by covalent bonds during splicing. Different from other RNAs, circular RNAs are well known due to their circular structure. In recent years, many researches were conducted to investigate the role of circular RNAs in multiple diseases. To better understand the structure of circular RNAs, we reviewed the biogenesis and related regulation at first. Mechanisms by which circular RNAs exert effects were summarized then. Due to the conserved and brain-specific characteristic, circular RNAs in brain were depicted next. At last, considering the high mortality rate and disability rate caused by stroke globally, we reviewed related articles and summarized the results of original articles. Circular RNAs are suggested to be involved in the pathogenesis of stroke as well as some other neurological diseases which provides new insights and potential targets in clinical application.

## 2. Introduction

Circular RNAs (circRNAs) are single-stranded RNAs which are closed by covalent bonds during splicing. circRNAs was discovered decades ago. However, because of the lack of free 3′ and 5′ ends which are different from linear RNA, circRNAs cannot be detected by molecular technology dependent on poly-adenylated (poly A) tail. Therefore, only a small amount of circRNAs are found. At first, circRNAs are regarded as the by-products of aberrant splicing, with almost no biological function. But powered by advanced high-throughput RNA sequencing in recent years, circRNAs are found to express in eukaryotic cells universally. At the same time, more and more studies start focusing on the function of circRNAs, which reveals

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that circRNAs are involved in gene regulation [1, 2].

According to the related current researches, the characteristic of circRNAs are as follows: ①Huge amount and widely expressed: High-throughput sequencing (HTS) reveals the universal expression of tens of thousands of circRNAs in Metazoa, from fruit flies and worms to rodents and humans; ②Highly stable: the circular structure prevents the circRNAs from the cleavage of branching enzymes and exonucleases [3]; ③Highly conserved: many circRNAs have highly conserved sequences that can be detected not only in humans but also in rodents [3]; ④Tissue-specific: researches show that the circRNAs are represented at a high level in neural tissues [1, 4, 5].

Stroke is a major cause of death and disability worldwide, particularly in developing countries [6]. Ischemic stroke (IS) and intracerebral hemorrhage (ICH) are two subtypes of stroke. IS occurs when blood supply to a certain area of the brain is obstructed or reduced, usually caused by a thrombus. ICH causes brain injury characterized by neuronal death and blood brain barrier breakdown mainly by two mechanisms. Primary brain injury was caused by the repression and destruction of hematoma to brain tissue while inflammation response and release of clots components cause secondary brain injury [7]. Currently, treatments for stroke are limited. Further understanding the pathophysiological mechanism of the development of stroke might provide a new and more effective way to diagnose stroke and improve outcomes. Most non-coding RNA based strategies and related researches mainly concentrate on long non-coding RNAs and microRNAs (miRNAs) [8, 9]. Recently, several researches suggested the roles of circRNAs in stroke.

In conclusion, circRNAs participate in neural regulation and stroke pathogenesis. This review summarizes the result of recent researches and reviews the biogenesis and function of circRNAs. Furthermore, we discussed the role of the circRNAs in the brain in order to highlight the significance of circRNAs in neural system. At last, we focused on the effect of circRNAs in stroke which would provide new target for diagnosis and treatment.

## 3. circRNAs biogenesis and its regulation

Based on the constituent components, circRNAs could be categorized into three types: circular exonic RNAs (ecircRNAs), circular intronic RNAs (ciRNAs) and exon-intron circular RNAs (EIciRNAs).

EcircRNAs, which consist of only exon sequences, are mainly expressed in cytoplasm. They are highly stable whose half-life are more than 48 hours. Bioinformatics analysis suggested common characteristic of cyclized exons, in which adjacent flanking introns contained more complementary ALU repeats than non-circularized exons [3]. There are two main biogenesis mechanisms: ①Direct back-splicing: Pairing between complementary sequences from the flanking introns promotes the formation of an exon loop in which the downstream 5’ splice donor site is joined to an upstream 3’ splice acceptor site. ②Exon skipping [10]: During the exon skipping process, a lariat structure including exons is formed, and then undergoes the internal splicing where introns are excised and ecircRNAs are generated. Most circRNAs under research are this type of circRNAs.

ciRNAs, composed of intron sequences, mainly locate in the nucleus. The processing depends on a conserved consensus motif which contains a 7 nt GU-rich element near the 5’ splice site and an 11 nt C-rich element close to the branch-point site [11]. There are three main biogenesis mechanisms: ①group II introns are excised as ciRNAs through the form of a 2’,5’-phosphodiester bond; ②group I introns undergo self-splicing starting with nucleophilic attack of exogenous guanosine (exoG) onto the 5’ splice site, followed by 3’-terminal guanosine ($\omega$G) nucleophilic attacking onto a phosphodiester bond close to the 5’-end of the intron. Exons are ligated, and group I introns are circularized; ③ full length ciRNAs are generated by the hydrolysis of the exon at the 3 ’splice site, followed by the $\omega$G nucleophilic attacking onto the 5’ splice site [10].

EIciRNAs, composed of exon and intron sequences, mainly present in the nucleus [12]. Derived from messenger RNA precursors (pre-mRNA), EIciRNAs is formed by back-splicing [1, 13], whose content is much lower than homologous mRNA.

Derived from mRNA precursor, circRNAs are transcribed by RNA polymerase II (Pol II), which is related with Pol II transcription elongation rate [14]. circRNAs biogenesis are mainly regulated by the cis-regulatory elements and trans-acting factors which control splicing [1]. It is noteworthy that both circRNAs and classical linear RNA are generated by pre-mRNA through post-transcriptional regulatory mechanisms, which suggests that there is a competitive relationship between the two during the generation [15]. In addition, RNA binding protein regulates the production of circRNAs. For example, muscleblind protein (MBL) can promote the cyclizing of the exon of MBL gene [15]; Quaking protein (QKI) can up-regulate the expression level of a variety of circRNAs during the process of epithelial-mesenchymal transition (EMT) [16].

## 4. Function of circRNAs

### 4.1 Function as miRNA sponges

Some ecircRNAs are expressed stably and are rich in contents. They have miRNAs binding sites, which can compete with mRNA and thus regulate gene expression [1, 3]. The most typical example is ciRS-7 [17], which is derived from cerebellar degeneration related 1 (CDR1). antisense transcript and is mainly found in the brains of humans and mice [18]. It contains over 60 conserved miR-7 binding

site [19]. ciRS-7 was highly expressed in the cytoplasm and can bind up to 20,000 miR-7 molecules per cell [19], which might affect the binding of miR-7 to its target mRNA. Reduced expression of ciRS-7 resulted in decreased expression of mRNAs which contain miR-7 binding sites, which confirmed that mRNAs compete with ciRS-7 for miR-7 binding sites [18, 19]. In addition, the function of ciRS-7 was conserved, and ciRS-7 expression in zebrafish affected the development of midbrain, whose effect was consistent with miR-7 knockdown [1, 19]. However, a recent article published in Science pointed out that after ciRS-7 knockout in mice, expression level of miR-7 in the cerebral cortex, hippocampus, cerebellum, and olfactory bulb significantly reduced, while the downstream target gene Fos was upregulated. Thus, it can be seen that the mechanism related to miRNA sponge function remains to be explored [20]. In addition, sex-determining region Y of testicular-specific circRNA (circSRY) contains 16 miR-138 binding sites in the mouse brain [18]. circZNF91, originating from zinc finger protein gene contains 24 miR-23 binding sites and 39 miR-296 binding sites [21]. Generally speaking, circRNA derived from genes containing repeat coding regions contains a series of conserved miRNA binding sites [22].

### 4.2 Gene transcription regulation

Different from ecircRNAs, ciRNAs and EIciRNAs mainly exist in the nucleus. However, its mechanism of transportation and retention in the nucleus is unclear. In human cells, ciRNAs localize near the host gene and combine with RNA Pol II during transcription, which increased the level of transcription through a mechanism of cis-regulation [23]. Studies have found that knockdown of ciRNAs would result in decreased expression of parental mRNA. Similarly, EIciRNAs can also interact with RNA Pol II. Knocking down EIciRNAs can also reduce the mRNA expression level of their parental gene. EIciRNAs associate with U1 small nuclear ribonucleoprotein (snRNP) through characteristic RNA-RNA interaction between EIciRNA and U1 small nuclear RNA (snRNA). Then the EIciRNA-U1 snRNP complexes may further communicate with the Pol II transcription complex at the promoters of parental genes, thereby increasing gene expression [12, 13]. circNOL10 was reported to promote transcription factor (TF) sex comb on midleg-like 1 (SCML1) expression by inhibiting ubiquitination [24].

### 4.3 Encode protein or peptide directly

circRNAs were originally considered as a special type of non-coding RNAs, but several researches have pointed out that circRNAs can be translated into proteins. In 1995, it was reported that eukaryotic ribosomes can start translation on circRNAs when the RNAs contain internal ribosome entry site (IRES) elements. Recently, a circRNA derived from the muscleblind locus was suggested to encode a protein in fly head when extracted by mass spectrometry [25]. CircZNF609 contained a 753-nt open reading frame (ORF), which was similar to linear transcripts, starting from AUG of the host gene and ending with a stop codon [26]. CircZNF609 interacted with polysomes, and then translated into a protein in a splicing-dependent and cap-independent manner [27]. Besides, circFBXW7 was highly expressed in the normal human brain. The spanning junction ORF in circ-FBXW7 driven by internal ribosome entry site encoded a novel protein, FBXW7-185aa, which is a functional protein. Increased expression of FBXW7-185aa in cancer cells was found to inhibit proliferation and cell cycle acceleration, while downregulation of FBXW7-185aa promoted malignant phenotypes both *in vivo* and *in vitro*. FBXW7185aa could also decrease the half-life of c-Myc by inhibiting USP28-induced c-Myc stabilization. Findings above suggested that circ-FBXW7 and FBXW7-185aa have potential prognostic significance in brain tumor [28]. Another novel tumor suppressor protein, SHPRH-146aa, consistent with the full-length protein and behaving as a protective molecule to decrease degradation, was derived from circ-SHPRH driven by IRES elements [29]. Besides, circ$\beta$-catenin could produce a novel 370-amino acids $\beta$-catenin isoform which shared the same start codon with the linear mRNA transcript but ended at a new stop codon when cyclizing. Silencing of circ$\beta$-catenin significantly suppressed malignant phenotypes both *in vivo* and *in vitro*, while knockdown reduced the protein expression level of $\beta$-catenin without influencing its mRNA level which further validates the possibility of circRNAs translation [30]. In addition, poly-adenosine or poly-thymidine in 3′ UTR could inhibit circRNAs translation [31] and m6A motif was found to initiate translation in endogenous circRNAs with translation potential in cellular responses to environmental stress [32]. The correlated mechanism is shown in Fig. 1.

### 4.4 Function as biomarkers

Considering circRNAs are stable and expressions are cell-specific, selectively abundant and varying in different diseases, circRNAs are expected to have the potential to be a biomarker [33]. circRNAs were identified in several kinds of fluids, including exosomes [34], saliva [35], and blood [36]. Actually, circRNAs were suggested to behave as biomarkers in a variety of diseases, including cancer [34], systemic lupus erythematosus [37], rheumatoid arthritis [38], diabetes [39] and tuberculosis [40]. In addition, in the condition of nervous system diseases, such as Alzheimer's disease (AD) [41], stroke [42] and amyotrophic lateral sclerosis (ALS) [43], blood-brain barriers are easy to be damaged, allowing free or exosome-encapsulated circRNAs [44] to release from the brain and be measured peripherally. Therefore, in neurological diseases, brain-specific circRNAs might be released into the circulatory system, so that the level of circRNAs in the blood can be used to monitor disease progression or achieve therapeutic purposes.

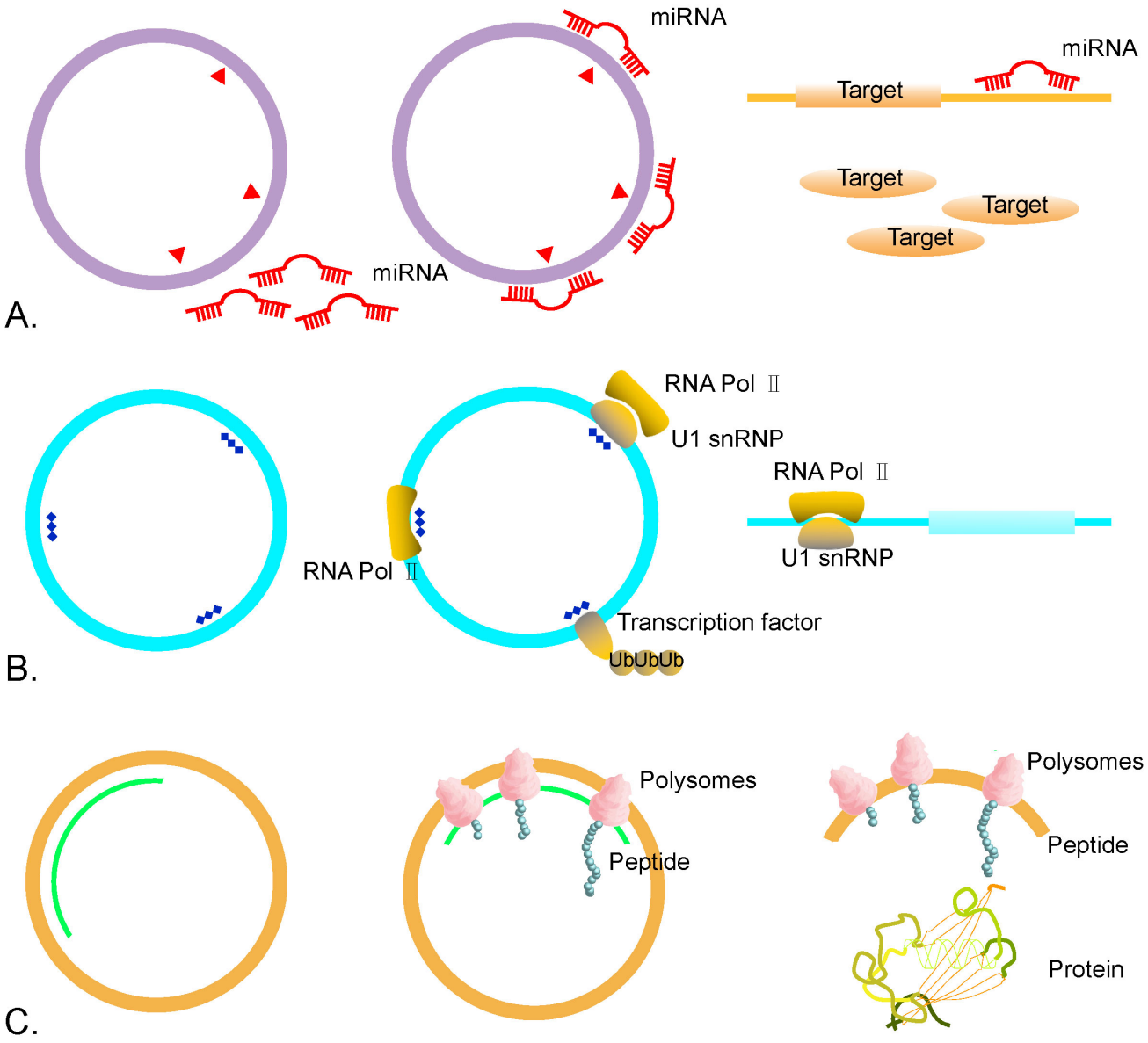


**Fig. 1. Three possible mechanisms that circRNAs exert functions**. (A). Function as miRNA sponges. circRNAs could interact with miRNAs and further relieve the suppression of target genes by miRNAs. (B). Gene transcription regulation. circRNAs have binding sites of RNA binding protein. They could interact with U1 snRNP, RNA Pol II or transcription factors and then regulate gene transcription. (C). Encode protein or peptide directly. circRNAs might transcript into peptides or proteins and proteins play a role directly.

## 5. circRNAs in the brain

circRNAs are conserved in sequence in the mammalian brain, either in humans and mice [45], and sometimes can be detected even in the brain of drosophila [5]. To comprehensively identify the tissue-specific expression of mammalian circRNAs, You *et al.* performed a thorough sequencing analysis of total RNA samples (excluding rRNA) from several mouse tissues (i.e., brain, liver, lung, heart, and testis). In all tissues they examined, circRNAs content was clearly the highest in brain. Either in the percentage of circular junction reads from all the reads mapped on the genome, or the percentage of genes that generated circRNAs from all the expressed genes, brain ranked the highest with 0.75–0.87% and 20–21%, respectively. Both higher exclusively expression of circRNAs host genes in brain [45, 46] and a higher contribution of circRNAs in brain when a host gene is universally expressed in many tissues lead to the higher enrichment of circRNAs in brain [46]. This illustrates that circRNAs are highly conserved and brain-specific, which provide the basis for performing research in rats or mice and reveal the validation and significance of conducting circRNAs-related study in brain.

In human fetal brain tissue, there are on average 8914 tissue-specific circRNAs per sample which is the most abundant in fetal tissues and suggests an important

role in brain development [45]. During neuronal differential, stable-state level of circRNAs significantly upregulated as indicated by their total number and expression while the level of linear mRNAs kept almost unchanged [14]. A research divided rhesus macaque into two age groups-adult (10-year) and aged (20-year) Totally, 17,050 well-expressed, stable circRNAs were found. Approximately 3% (475/17050) age-different circRNAs, including 272 20y-specific and 203 10y-specific, were found during brain aging [47] which indicates an aged accumulation of circRNAs in aging brain [47, 48]. Besides, eight anatomical brain compartments were deeply sequenced. Cerebellar circRNAs are different from that of other areas which revealed a spatial-specific characteristic [47]. Sex-biased expression of circRNAs was also found in rhesus macaque brain which is similar to protein differential expression in human brain [47].

In addition, studies have suggested that circRNAs are enriched in synaptosomes [5, 46]. High-resolution *in situ* hybridization was used for validation while circRNAs were visualized in cultured hippocampal neurons. CircHomer1_a is a circRNA generated from the synaptic scaffolding molecule Homer1 transcript. The result of *in situ* hybridization revealed abundant expression of circHomer1_a in both neuropil and somata layers of CA1 hippocampal region. Besides, many circRNAs upregulate their expression levels abruptly during synaptogenesis. During development of hippocampus, upregulated circRNAs were generated from the gene loci that also coded for proteins enriched with synapse-related functions. Results of high-throughput sequencing, quantitative reverse transcription polymerase chain reaction (qRT-PCR) and *in situ* hybridization are consistent with each other. All indicated that circRNAs expression is developmentally regulated [46]. However, the regulation of circRNAs was independent of their host genes which indicate the specific role of circular structure. In summary, a change in the expression pattern for many circRNAs related with the beginning of synaptogenesis was observed which suggests a role of circRNAs in development of hippocampus.

## 6. circRNAs and stroke

### 6.1 circRNAs and IS

Many studies have reported circRNAs microarrays or sequencing results which are related with IS. After validation by qRT-PCR, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed. Several researches were conducted in patients and healthy controls while the others used rodent models or cells. In human sample, the ratio of serum hsa-circRNA-284 and miR-221 was found to notably increase in the patients of IS within 5 days ($P = 0.0002$), which had a biomarker potential of carotid plaque rupture and stroke [49]. Ostolaza *et al.* found that a set of 60 circRNAs were increased in atherotrombotic compared with cardioembolic strokes. Differential expression of hsa-circRNA-102488 which is originated from UBA52 gene, was further validated. This suggests that circRNA might be a new source of biomarker for IS etiology [50]. Moreover, circFUNDC1, circPDS5B and circCDC14A were increased in IS patients versus healthy controls and were reported to serve as diagnostic and prognostic biomarkers [51]. In addition, 373 circRNAs were increased, while 148 were decreased when compared the peripheral blood mononuclear cells (PBMCs) from IS patients versus healthy controls. Thirteen candidate circRNAs were verified by qRT-PCR. Bioinformatics analysis showed that aberrantly expressed circRNAs were associated with inflammation and immunity which were involved in IS pathogenesis and had the potential to be diagnostic biomarker [52]. While using rodent models, 128, 198, and 789 circRNAs were found to be significantly changed at 5 min, 3 h, and 24 h after IS in mice blood sample. After validation by IS patient blood sample, hsa-circ-0090002 and hsa-circ-0039457 were reported to be differentially downregulated [53]. Besides, after 45-minute-transient middle cerebral artery occlusion (MCAO), circRNA microarrays revealed that 1027 circRNAs were significantly changed in 48 hours after reperfusion in the ischemic brain versus the sham group in which 914 circRNAs were notably increased, while the other 113 were notably decreased. The expression level of mmu-circRNA-40001, mmu-circRNA-013120, and mmu-circRNA-40806 was further validated by qRT-PCR. After GO and KEGG analyses, the Rap1 and Hippo signaling pathway which are involved in cell survival and death regulation was found to be the most enriched [54]. In another microarray analysis, 283 circRNAs were changed (>2-fold) at least at one of the reperfusion time points, whereas 16 were changed at all 3-time points (6 h, 12 h and 24 h) of reperfusion after transient MCAO studied versus sham. Then bioinformatics analysis showed that binding to proteins, ions and nucleic acids, cell communication, metabolic process, and biological regulation are leading biological and molecular functions regulated by circRNAs change after transient MCAO [55]. Duan *et al.* suggested that totally 14,694 circRNAs from 6 brain tissues were detected differentially expressed by HTS of which 87 showed a significant fold change >2 ($P < 0.05$). rno-circRNA-17737, rno-circRNA-8828 and rno-circRNA-14479 were significantly upregulated, while rno-circRNA-1059, rno-circRNA-9967 and rno-circRNA-6952 were significant downregulated when validated [56]. Zhang *et al.* reported that 16 and 28 circRNAs showed significant increase and decrease respectively in rat ischemia-reperfusion model by HTS. In *in vitro* experiments, levels of the hsa-circ-camk4 were elevated in SH-SY5Y cells exposed to oxygen-glucose deprivation/reoxygenation (OGD/R) treatment. Overexpression of hsa-circcamk4 in SH-SY5Y cells significantly in-

creased cell death rate after OGD/R. Bioinformatics analysis showed that circcamk4 was involved in the apoptosis signaling pathways, glutamatergic synapse pathway and MAPK signaling pathway, all of which are known to be included in brain injury after ischemia-reperfusion (I/R). This suggested that hsa-circ-camk4 may have been involved in in progression of cerebral I/R injury [57]. In parallel, 3 circRNAs were significantly increased, and 12 were decreased in HT22 cells with OGD/R compared with normal controls. Furthermore, upregulation of mmu-circRNA-015947 was validated by qRT-PCR. Bioinformatics analysis revealed that mmu-circRNA-015947 could interact with mmu-miR-188-3p, mmu-miR-329-5p, mmu-miR-3057-3p, mmu-miR-5098 and mmu-miR-683 and thus increase target gene expression. KEGG pathway analysis predicted that mmu-circRNA-015947 might be involved in apoptosis, metabolism and immune-related pathways, which are known to play a role in cerebral I/R injury [58]. The detailed information summary is shown in Table 1 [49–58]. Besides, circHECTD1 expression was upregulated in IS recurrence patients versus non-recurrence patients (160 : 160). Further, ROC curve analysis indicated that circHECTD1 expression predicted higher risk of IS recurrence. CircHECTD1 also correlated with higher disease risk, disease severity and inflammation [59]. Similar results were found in peripheral blood mononuclear cell circDLGAP4. CircDLGAP4 is decreased and negatively related with severity, inflammatory cytokine expression such as TNF-$\alpha$, IL-6, IL-8 as well as IL-22 and miR-143 expression in IS patients [60]. Moreover, *in vitro*, silencing of circHIPK2 stimulated neural stem cells (NSCs) specifically differentiated into neurons but made no difference to the differentiation to astrocytes. *In vivo*, microinjection of si-circHIPK2-NSCs could migrate to the ischemic area after stroke induction. Si-circHIPK2-NSCs increased neuronal plasticity in the ischemic brain, led to long-lasting neuroprotection, and significantly reduced functional dysfunction which exhibits a promising therapeutic strategy to neuroprotection [61]. The findings suggest that circRNAs might be a biomarker for IS diagnosis and involved in IS pathogenesis but the concise mechanism needs further investigation.

When it comes to the mechanism research, some studies indicate that circRNAs are involved in the process of IS by interacting with miRNAs and subsequent downstream target genes. CircDLGAP4 was found to be downregulated in IS patients and tMCAO mice and functioned as miR-143 sponge to repress miR-143 activity, which inhibited the expression of homologous to the E6-AP C-terminal (HECT) domain E3 ubiquitin protein ligase 1 (HECTD1). Overexpression of circDLGAP4 enhanced tight junction protein expression and decreased mesenchymal cell marker expression which inhibited endothelial-mesenchymal transition (EMT). and would lead to blood brain barrier (BBB). protection [62]. In parallel, circHECTD1 was upregulated in tMCAO mice and functioned as an endogenous miR-142 sponge to repress miR-142 activity, resulting in the inhibition of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) inducible poly (ADP-ribose) polymerase (TIPARP) expression. The knockdown of circHECTD1 reduced infarct volumes and attenuated neurological deficits in transient MCAO mice through regulation of astrocyte activation via inhibition of autophagy [63]. Another associated study illustrated that circTLK1 functioned as an endogenous miR-335-3p sponge to repress miR-335-3p activity, which resulted in the increasing expression of TIPARP and a subsequent exacerbation of neuronal injury. The knockdown of circTLK1 significantly decreased infarction area, ameliorated neuronal injury, and improved neurological dysfunction [64]. Results are consistent with and complement each other, which provide new potential therapeutic targets for IS patients.

### 6.2 circRNAs and ICH

In ICH model, SD rats were injected with autologous artery blood. 111,1145,1751 circRNAs were upregulated while 47,732,1329 were downregulated in the cerebral cortex of ICH rats at 6 h, 12 h and 24 h after ICH induction compared with sham group (fold change $\geq$ 1.5, *P*-value $\leq$ 0.05). 93 were up-regulated and 20 were down-regulated at all three time points. The expression level of 6 circRNAs were further validated using qRT-PCR while 2 up-regulated circRNAs (rno_circRNA_011054 and rno_circRNA_005098) and 1 down-regulated circRNA (rno_circRNA_012556) were statistically altered. After GO and KEGG analyses, parent genes showed transition from protein complex assembly, cell-cell adhesion and cyclic adenosine monophosphate (cAMP) signaling pathway at 6 h to intracellular signal transduction, protein phosphorylation and glutamatergic synapse at 12 h and 24 h. Enrichment analyses of targeted mRNAs indicated transcriptional regulations and pathways including Ras-associated protein 1 (Rap1), mitogen-activated protein kinase (MAPK), phosphatidylinositol 3 kinase/protein kinase B (PI3K-Akt), tumor necrosis factor (TNF) and Wingless/Integrated (Wnt) signaling pathways in cancer [65]. Findings above suggest an important role of circRNAs in ICH and provide potential mechanism direction for further basic research and a promising target for ICH treatment.

circRNAs also participated in the pathogenesis of other neurological diseases, such as AD, Parkinson's disease (PD), motor neuron disease (MND) and demyelination diseases. In AD, many researches also focused on expression profiles [66–72]. Consistent with result above, majority of AD-associated circRNAs are independent from changes in cognate linear mRNA expression [66]. circRNAs are found to play roles in AD [20, 73–77], either by interacting with proteins [73] or miRNAs [74, 75]. In PD, circDLGAP4 exerted neuroprotective effects via miR-134-5p/CREB pathway [78] while circzip-2 possible sponged

**Table 1. Detailed information summary of circRNAs expression profiles in IS.**

| Study no. | Species-Model | Microarray or sequence sample | Differentially expressed circRNAs (Fold change, *P*-value). | Validation sample | Differentially expressed circRNAs (Fold change, *P*-value). | GO enrichment and KEGG pathway analysis | Interaction network of circRNA-miRNA-target genes | Significance |
|---|---|---|---|---|---|---|---|---|
| 1 [49] | Homo sapiens-Human | 24 asymptomatic: 17 urgent symptomatic IS patients serum | circR-284: miR-221, elevated in the urgent group ($P$ = 0.0002). | 47 asymptomatic, 41 urgent[a] patients, 24 other symptomatic[b] patients serum | circR-284: miR-221, elevated in the urgent group ($P < 0.001$). | - | - | serum circR-284: miR-221 might act as a diagnostic biomarker of carotid plaque rupture and stroke |
| 2 [50] | Homo sapiens-Human | 30 stroke patients with different stroke etiologies | 60 circRNAs upregulated and 159 circRNAs downregulated (FC $\geq$ 1.5, *P*-value $\leq$ 0.05). In atherothrombotic: cardioembolic stroke patients venous blood sample; 87 circRNAs upregulated and 139 circRNAs downregulated in atherothrombotic: undetermined strokes (FC $\geq$ 1.5, *P*-value $\leq$ 0.05); 8 circRNAs were found to be upregulated and 9 circRNAs were downregulated in cardioembolic: undetermined strokes | 25 atherothrombotic: 25 cardioembolic IS patients samples | Hsa-circRNA-102488 was significantly downregulated in atherothrombotic stroke patients compared with cardioembolic samples when normalizing to GAPDH mRNA (*P*-value $< 0.01$), as well as to the corresponding UBA52 mRNA (*P*-value $< 0.001$) ($P < 0.001$) | RNA-, ion- and enzyme-binding molecules, a protein and nucleic acid binding transcription factor activity and to be involved in cellular nitrogen compound metabolic, biosynthetic, cellular protein modification and gene expression processes; fatty acid biosynthesis and metabolism, extracellular matrix-receptor interaction, lysine degradation or arrhythmogenic right ventricular cardiomyopathy | Hsa-circRNA-102488-following miRNAs: hsa-miR-1182, hsa-miR-1299, hsa-miR-431, hsa-miR-516b, hsa-miR-668 and hsa-miR-766 | a new source of biomarkers of stroke etiology |
| 3 [51] | Homo sapiens-Human | 3 IS patients: 3 matched healthy controls | 10 were up-regulated and 68 were down-regulated circRNAs (fold changes $\geq$4 and *P*-values $< 0.05$) | Validation: 36 IS patients: 36 matched controls; Replication: 200 IS patients: 100 matched controls | circFUNDC1, circPDS5B and circCDC14A were upregulated in IS patients compared with healthy controls | - | - | (1) 3 circRNAs levels were positively correlated with infarct volume, suggesting as potential biomarkers for AIS diagnosis; (2) change rate in circRNAs within the first 7 days of treatment could serve as a potential biomarker for predicting stroke outcome; (3) Elevations of circPDS5B and circCDC14A in plasma might be derived from lymphocytes and granulocytes |

**Table 1. Continued.**

| Study no. | Species-Model | Microarray or sequence sample | Differentially expressed circRNAs (Fold change, *P*-value) | Validation sample | Differentially expressed circRNAs (Fold change, *P*-value) | GO enrichment and KEGG pathway analysis | Interaction network of circRNA-miRNA-target genes | Significance |
|---|---|---|---|---|---|---|---|---|
| 4 [52] | Homo sapiens-Human | 5 IS patients: 5 healthy controls peripheral blood mononuclear cells | 373 circRNAs were upregulated and 148 were downregulated | 5 IS patients: 5 healthy controls peripheral blood mononuclear cells | four were upregulated and four were downregulated | The pathways involved include the metabolic pathways, the mitogen activated protein kinase (MAPK). signaling pathway, some inflammatory pathways (e.g., the nuclear factor κ B (NF κB). signaling pathway, the tumor necrosis factor (TNF). signaling pathway, and the chemokine signaling pathway), immune pathways (e.g., the Toll-like receptor signaling pathway), and some signaling pathways associated with cell proliferation, differentiation, and apoptosis (e.g., the phosphatidylinositol 3 kinase/protein kinase B signaling pathway, the Ras related protein 1 signaling pathway, the FoxO signaling pathway, and the Janus kinase signal transducer and activator of transcription signaling pathway); Processes of three domains: biological process, cellular component and molecular function | 8 circRNAs-miRNAs | circRNAs in PBMCs[c] may be diagnostic biomarkers or potential therapeutic targets |

**Table 1. Continued.**

| Study no. | Species-Model | Microarray or sequence sample | Differentially expressed circRNAs (Fold change, *P*-value) | Validation sample | Differentially expressed circRNAs (Fold change, *P*-value) | GO enrichment and KEGG pathway analysis | Interaction network of circRNA-miRNA-target genes | Significance |
|---|---|---|---|---|---|---|---|---|
| 5 [53] | Mus musculus-Mice | 5 min, 3 h and 24 h MCAO[d] sham | 1051, 782, and 2721 circRNA probes exhibited differential expression at 5 min, 3 h, and 24 h of MCAO | Verification by MCAO ischemia brain tissue; Validation by IS patients and matched controls blood sample | hsa_circ_0090002 (PHKA2) and hsa_circ_0039457 (BBS2) were downregulated (*P* = 0.001 and 0.01 respectively). | circRNAs parental genes: 5 min MCAO, the major pathways include platelet-derived growth factor (PDGF), insulin, focal adhesion, and chemokine signaling pathways. 3 h MCAO, the major pathways include glutamatergic synapse, calcium signaling, and GnRH signaling. 24 h MCAO, the identified pathways include glutamatergic synapse, calcium signaling, and vascular smooth muscle contraction. CircRNAs-miRNAs-Target Genes: 5 min MCAO, the major biological functions were antioxidant, catalytic, receptor, signal transducer, and transporter activities; 3 h MCAO, most gene functions were involved in the regulation of catalytic activity, while genes involved in antioxidant activity were prominently increased; 24 h MCAO, most gene functions were associated with binding and catalytic activity; The stroke-related biological processes included the immune process, metabolic process, and biological adhesion. The significant pathways after 5 min MCAO were associated with the Hippo signaling pathway, cytokine-cytokine receptor interaction, metabolism of xenobiotics by cytochrome P450, biosynthesis of unsaturated fatty acids, gap junction, and mucin type O-glycan biosynthesis; At 3 h, extracellular matrix (ECM). -receptor interaction, metabolism of xenobiotics by cytochrome P450, and glycosphingolipid biosynthesis of lacto- and neolacto-series were identified. At 24 h, most of the identified significant pathways were associated with fatty acid metabolism | 24 circRNAs highly altered after ischemia at the three time points- miRNAs | circBBS2 and circPHKA2 may serve as potential biomarkers for IS diagnosis |

**Table 1. Continued.**

| Study no. | Species-Model | Microarray or sequence sample | Differentially expressed circRNAs (Fold change, *P*-value) | Validation sample | Differentially expressed circRNAs (Fold change, *P*-value) | GO enrichment and KEGG pathway analysis | Interaction network of circRNA-miRNA-target genes | Significance |
|---|---|---|---|---|---|---|---|---|
| 6 [54] | Mus musculus-Mice | transient MCAO (45 min ischemia and reperfusion at 48 h): sham brain tissue | 914 upregulated, 113 downregulated (fold change $\geq$2; $P < 0.05$) | transient MCAO: sham | mmu-circRNA-40001 (fold change = 3.40, $P < 0.001$), mmu-circRNA-013120 (fold change = 2.43, $P = 0.002$). Upregulated, mmu-circRNA-40806 (fold change = 0.418, $P < 0.001$). Downregulated | Rap1 signaling pathway and the Hippo signaling pathway, which regulate cell survival and death | 3 circRNAs-13 miRNAs-corresponding genes, which are related with brain injury and recovery | circRNAs are extensively involved in brain injury after stroke |
| 7 [55] | Mus musculus-Mice | 3/group transient MCAO (90 min ischemia and reperfusion at 6 h, 12 h, and 24 h: 3 sham penumbral cortex | 283 were altered at least at one of the reperfusion time points, 16 were altered at all 3-time points of reperfusion (fold change $\geq$2) | 3 transient MCAO (90 min ischemia and reperfusion at 6 h: 3 sham penumbral cortex | circ-008018, circ-015350, circ-016128 upregulated; circ-011137, circ-001729, circ-006696 downregulated at 6 h reperfusion | MAPK signaling (9 genes), cell cycle (7 genes), regulation of actin cytoskeleton (6 genes). and focal adhesion (5 genes) | circ-008018-353 miRNAs; circ-015350-80 miRNAs; circ-016128-86 miRNAs; circ-001729-72 miRNAs; circ-006696-378 miRNAs | biological regulation, metabolic process, cell communication, response to stimulus |
| 8 [56] | Rattus norvegicus-Rats | MCAO: control brain tissue | 4745 circRNAs were identified in both the MCAO and the control group while 4630 were only identified in control group and 4319 were only identified in MCAO group; 40 were up-regulated and 47 were down-regulated circRNAs (fold changes $\geq$2 and *P*-values $< 0.05$) | MCAO: control brain tissue | circRNA-17737, circRNA-8828, circRNA-14479, upregulated; circRNA-1059, circRNA-9967, circRNA-6952 downregulated | The top significant biological process term was "nervous system development (GO: 0007399)". The top enriched cellular component terms was implicated in "cytoplasmic vesicle membrane (GO: 0030659)". The top significant molecular function was "phosphatidylinositol binding (GO: 0035091)". The most significant pathways were referred to "Phosphatidylinositol signaling system (rno04070)," "Transcriptional misregulation in cancer (rno05202)" and "Endocytosis (rno04144)" | CircRNA-miRNA network | Needs further validation |

**Table 1. Continued.**

| Study no. | Species-Model | Microarray or sequence sample | Differentially expressed circRNAs (Fold change, *P*-value) | Validation sample | Differentially expressed circRNAs (Fold change, *P*-value) | GO enrichment and KEGG pathway analysis | Interaction network of circRNA-miRNA-target genes | Significance |
|---|---|---|---|---|---|---|---|---|
| 9 [57] | Rattus norvegicus-Rats; Rattus norvegicus-primary neuronal cells; Homo sapiens-SH-SY5Y cells | 4 MCAO rats: 4 control cerebral cortex | 16 cirRNAs upregulated 28 circRNAs downregulated | 3 h, 6 h OGD/R: normal controls | hsa-circ-camk4 are elevated | glutamatergic synapse pathway, MAPK signaling pathway, and apoptosis signaling pathways | Circ-camk4- rno-miR-27a-3p, rno-miR324-3p, rno-miR-212-3p and rno-miR-504-69 target genes | circ-camk4 may play a key role in progression of cerebral I/R injury |
| 10 [58] | Mus musculus-HT22 cells | OGD/R: normal controls | 3 upregulated, 12 downregulated | OGD/R: normal controls | mmu-circRNA-015947 was upregulated (fold change = 1.64, *P* = 0.01). | apoptosis-related, metabolism-related and immune-related pathways | mmu-circRNA-015947-mmu-miR-188-3p, mmumiR-329-5p, mmu-miR-3057-3p, mmu-miR-5098 and mmu-miR-683 | mmu-circRNA-015947 might be involved in the process of cerebral ischemia-reperfusion injury |

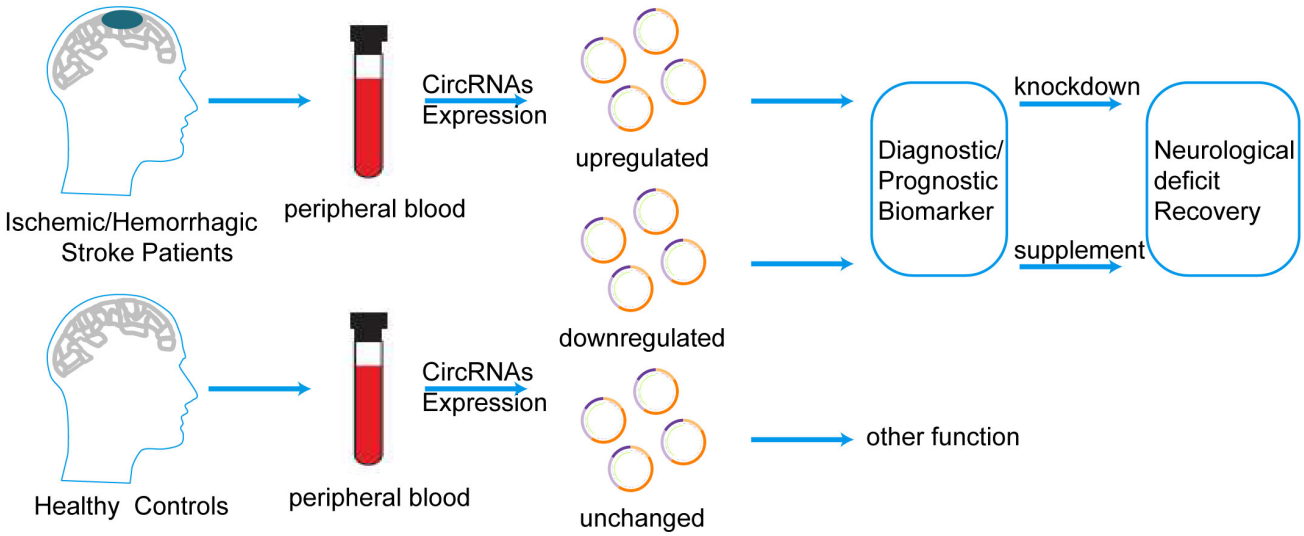


**Fig. 2. The roles of circRNAs in stroke.**

miR-60-3p [79]. Similar to other diseases, expression profile was detected by RNA-sequencing in different brain regions of PD mouse model, not comparison between case group and control group [80]. circRNAs are also suggested to behave as biomarkers in ALS [81]. Moreover, in demyelinating diseases, circRNAs either in exosomes [82] or in peripheral blood leucocytes [83] could be used as biomarkers. The expression level of ecircRNAs were significantly decreased in peripheral blood mononuclear cells of relapsing-remitting multiple sclerosis patients compared to controls [84].

## 7. Perspective

Up to now, the field of circRNAs research is relatively limited but rapidly developing. The research methods and databases of circRNAs have gradually matured with the efforts of global researchers. The function of circRNAs has also been gradually expanded around the molecular sponge. circRNAs were proposed as non-coding RNAs from the beginning, but now are found to encode proteins. circRNAs also provide new detection methods and treatment approaches for several clinical diseases due to its tissue specificity and biological stability.

High expression and conservation of circRNAs in the brain should draw our attention. Many researches also indicate the part of circRNAs in the development and aging of brain. Enrichment in synaptosomes suggested that circRNAs might be involved in synaptic regulation. Stroke has the highest incidence of neurological diseases which has a high fatality rate and disability rate, increasing the social burden. However, researches related with stroke are limited while ICH researches are even fewer. Most original studies related with the role of circRNAs in stroke revealed an upregulation or downregulation. Differential expression reveals its feasibility as a biomarker, either as a diagnostic or prognostic biomarker. At present, serum is widely used as clinical samples. However, due to the specificity of neural system, we could pay more attention to cerebrospinal fluid. After microarray or sequencing, expression profile is carried out and then bioinformatics analysis such as GO enrichment and KEGG pathway is performed. These results predict the function and mechanism of circRNAs and provide a basis for further research. Validation of the function of circRNAs focuses on miRNA sponge. We also speculate that circRNAs might exert the function by interacting with genes or encode proteins directly. The roles of circRNAs in stroke were depicted in Fig. 2. With the deepening of research, we assume that in the near future we could reveal the function and mechanism of circRNAs in stroke, and further turn basic research into clinical application. It is well founded to speculate that circRNAs might be a potential therapeutic target.

## 8. Author contributions.

HZ and ZH propose the outline. YW searched the PubMed for relevant papers and wrote the manuscript. YW revised the paper.

## 9. Ethics approval and consent to participate

Not applicable.

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## 12. Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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**Abbreviations:** BBB, blood brain barrier; cAMP, cyclic adenosine monophosphate; CDR1, Cerebellar degeneration related 1; circRNAs, Circular RNAs; circSRY, Sex-determining region Y of testicular-specific circRNA; ciRNAs, Circular intronic RNAs; ecircRNAs, Circular exonic RNAs; EIciRNAs, Exon-intron circular RNAs; EMT, epithelial-mesenchymal transition; exoG, Exogenous guanosine; GO, Gene Ontology; HECTD1, homologous to the E6-AP C-terminal domain E3 ubiquitin protein ligase 1; HTS, High-throughput sequencing; ICH, Intracerebral hemorrhage; IS, Ischemic stroke; IRES, Internal ribosome entry site; I/R, ischemia-reperfusion; KEGG, Kyoto Encyclopedia of Genes and Genomes; MAPK, mitogen-activated protein kinase; MBL, Muscleblind protein; MCAO, middle cerebral artery occlusion; miRNAs, microRNAs; MND, motor neuron disease; NSCs, neural stem cells; OGD/R, oxygen-glucose deprivation/reoxygenation; PBMCs, peripheral blood mononuclear cells; PI3K-Akt, phosphatidylinositol 3 kinase/protein kinase B; Pol II, polymerase II; poly A, Poly-adenylated; pre-mRNA, messenger RNA precursors; QKI, Quaking protein; qRT-PCR, quantitative reverse transcription polymerase chain reaction; Rap1, Ras-associated protein 1; SCML1, Sex comb on midleg-like 1; snRNA, small nuclear RNA; snRNP, small nuclear ribonucleoprotein; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; TF, Transcription Factor; TIPARP, poly [ADP-ribose] polymerase; TNF, tumor necrosis factor; Wnt, Wingless/Integrated; $\omega$G, terminal guanosine.

**Keywords:** Review; Circular RNAs; Stroke; Biomarker; miRNA sponge

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