Editorial

Circular RNA as a prospective molecular tool for the study of neuroprotection in cerebral ischemia

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We recently read the article by Han *et al.* [2018]: "Novel insight into circular RNA *HECTD1* in astrocyte activation via autophagy by targeting MIR142-*TIPARP*: implications for cerebral ischemic stroke" published in *Autophagy* (1). This study reported that circular RNA of *HECTD1* gene (*circHECTD1*) is upregulated in the blood of patients with acute ischemic stroke (AIS) and in animal and cell line ischemia model conditions. Simultaneously, knockdown of *circHECTD1* reduced infarct areas and attenuated neuronal deficits and astrocyte activation in a mouse model of transient middle cerebral artery occlusion (tMCAO). Han *et al.* [2018] showed that *circHECTD1* functions as an endogenous microRNA sponge to inhibit MIR142 activity, resulting in the inhibition of TCDD-inducible poly[ADP-ribose] polymerase (*TIPARP*) gene expression with subsequent inhibition of astrocyte activation via autophagy. In conclusion, the authors hypothesized that *circHECTD1* can serve as a novel biomarker and therapeutic target for stroke (1).

Here, we want to emphasize the significance of the investigation by Han *et al.* [2018] as an important step towards the development of new approaches in understanding the pathogenesis of stroke. We also discuss the role of circular RNAs in the diagnosis and treatment of cerebral ischemia and comment on a number of research articles that preceded Han *et al.* [2018] in this field of study.

Undoubtedly, the problem of vascular diseases of the brain and, in particular, ischemic stroke, retains its medical and social significance due to the high rates of morbidity, disability and mortality of this disease in the world (2). To date, much attention has been paid to the study of specific types of RNAs, as markers in diagnosis and as targets in the treatment of ischemic stroke. It is well known that cerebral ischemia causes a cascade of biochemical and transcriptome changes in brain tissues (3). It has been shown that reperfusion after ischemia causes additional damage in brain cells, including the destruction of the endothelial cells of the microvascular brain, disturbance of the blood-brain barrier, accumulation of excess oxygen radicals and activation of apoptosis (4,5). Recently, in a rat model of tMCAO, we revealed the activation of a large number of genes involved in inflammation, the immune response, apoptosis and the stress response (6). Simultaneously, a massive downregulation of genes that ensure the functioning of neurotransmitter systems was observed in tMCAO conditions.

To date, it has been shown that not only messenger RNA (mRNA) but also various types of regulatory RNA are involved in the response to the pathological effects (7-10). Of particular interest are circular RNAs (circRNAs), which have a covalently closed structure and are often formed in protein-coding genes during back-splicing (11,12). circRNAs demonstrate an increased resistance to the action of exonucleases and a predominantly brain-specific expression pattern (13-15). However, the functional significance of circRNA is not well understood. Currently, great attention is being paid to the study of the functioning of circRNAs as microRNA (miRNA) sponges. CircRNA acting as competitive endogenous RNA (ceRNA) competes with mRNA for binding to miRNA and diminishing the effect of miRNA on transcriptional and post-transcriptional levels of regulation of gene expression (16,17). The

Table 1 Circular RNAs associated with ischemia conditions

Circular RNA	Ischemia conditions	Confirmation of specific expression	Confirmation of miRNA-circRNA interaction	Neuroprotective effect	Reference
circHECTD1	tMCAO in mice, plasma of AIS patients, human glioblastoma A172 cell line treated with OGD-R	Microarrays and real-time RT-PCR	Luciferase activity assays	Knockdown are associated with neuroprotection	Han *et al.* [2018]
circDLGAP4	tMCAO in mice, plasma of AIS patients, mouse brain endothelial bEnd.3 cells treated with OGD-R	Real-time RT-PCR	Luciferase activity assays, miRNA pull-down and FISH assays	Upregulation are associated with neuroprotection	Bai *et al.* [2018]
circ_016128, circ_007362, circ_006839, circ_000113, circ_002664, circ_008018, circ_011381, circ_015350, circ_006696, circ_001729, circ_000741, circ_016423, circ_009396, circ_017370, circ_010383, circ_016289	tMCAO in mice	Microarrays and real-time RT-PCR	Computational prediction	–	Mehta *et al.* [2017]
mmu_circRNA_40001, mmu_circRNA_013120, mmu_circRNA_40806	tMCAO in mice	Microarrays and real-time RT-PCR	Computational prediction	–	Liu *et al.* [2017]
mmu-circRNA-015947	HT22 hippocampal cell culture treated with OGD-R	Microarrays and real-time RT-PCR	Computational prediction	–	Lin *et al.* [2016]

tMCAO, transient middle cerebral artery occlusion.

functions of several circRNAs as miRNA sponges were investigated in various pathologies. In particular, the role of circRNA CIRs-7 in preventing neuropsychiatric disorders in mice, associated with its functioning as a ceRNA, was recently established (17). In addition, in Alzheimer's disease (18) and various types of cancer (19-21), results showing that circRNA-miRNA-mRNA competition may be associated with pathogenesis regulation were obtained.

In the last 2 years, studies have investigated the transcriptional profile and functional properties of circRNAs under conditions simulating cerebral ischemia (see *Table 1*). Recently, in HT22 hippocampal cell culture under conditions of oxygen glucose deprivation-reoxygenation (OGD-R), which simulated damage after cerebral ischemia and reperfusion, Lin *et al.* [2016] showed that circRNAs may function as miRNA sponges (22). In the OGD-R model, it was shown that circRNA expression is associated with the metabolic pathways of apoptosis and immune response. In tMCAO model conditions, Mehta *et al.* [2017] showed that the differentially expressed circRNAs might be controlled by a set of transcription factors in stroke. The bioinformatics analysis of biological and molecular functions showed that circRNAs altered their expression after tMCAO controlled biological regulation, the metabolic process, cell communication, and binding to proteins, ions and nucleic acids (23). Simultaneously, it was shown that 16 circRNAs contain binding sites for many miRNAs. In addition, in the tMCAO model conditions in mice, Liu *et al.* [2017] (24) detected the changes in the expression of more than one thousand circRNAs using microarray analysis. These circRNAs were associated with signalling pathways regulating the processes of cell survival and death. Moreover, Liu *et al.* [2017] predicted possible interactions between circRNAs and miRNAs, which can provide potential information for revealing the mechanisms of brain damage in ischemic stroke. Thus, in the studies by Lin *et al.* [2016], Liu *et al.* [2017] and Mehta *et al.* [2017], the transcriptional profile of circRNAs was studied in detail under the conditions of the individual ischemia model. CircRNAs that most significantly altered the expression during ischemia were identified. However, the association with metabolic pathways and biological processes in the cell, as well as with miRNA interaction, was

mainly predictive and computational. Thus, further studies of circRNAs functioning as miRNA sponges in ischemia conditions are needed for specific examples.

A recent study published new important information on the functioning of circRNAs in ischemia. Bai *et al.* [2018] showed that the circRNA of *DLGAP4* gene (*circDLGAP4*) functions as an miRNA sponge to inhibit MIR143 activity, resulting in the inhibition of homologous to the E6-AP C-terminal domain E3 ubiquitin protein ligase 1 expression (25). In contrast, *circDLGAP4* levels were significantly decreased in the plasma of AIS patients and in a mouse stroke model. It has been shown that upregulation of *circDLGAP4* expression significantly attenuated neurological deficits, and decreased infarct areas and blood-brain barrier damage in the tMCAO mouse stroke model. From our perspective, several important conclusions can be immediately drawn from this study. First, blood is widely used in human diagnostic procedures, and the confirmation that transcription pattern in peripheral blood cells can reflect what occurs in the brain is of great significance. Second, the study clearly indicates that the regulation of the regenerative mechanisms of brain cells during ischemia occurs with the participation of circRNA, and in this case, is associated with upregulation of *circDLGAP4* expression. Third, the study by Bai *et al.* emphasizes the role of circRNA-miRNA-mRNA interactions in ensuring the regulation of pathogenesis and regeneration in ischemia.

In studying the functioning of circRNAs as miRNA sponges in cerebral ischemia, we next considered the work by Han *et al.* [2018] on novel insights into *circHECTD1* functioning in cerebral ischemia (1). In this paper, the authors hypothesized that upregulated *circHECTD1* directly binds to miRNA MIR142 and acts as an endogenous sponge to inhibit MIR142 activity, which results in astrocyte activation and thus contributes to cerebral infarction. First, the authors convincingly showed that *circHECTD1* is upregulated in mouse brain in tMCAO model conditions and in human glioblastoma A172 cell line treated with OGD-R. Han *et al.* [2018] clearly demonstrated that the expression pattern of *circHECTD1* in the blood of patients with AIS and in ischemia model conditions is similar. Second, they showed that the knockdown of *circHECTD1* was associated with the reduction of brain infarction in tMCAO mice. Consequently, *circHECTD1* participates in the regulation of the injury and regenerative mechanisms of brain cells in conditions of ischemia. Third, it was shown that in interaction with MIR142, which negatively affects the level of mRNA of *TIPARP* gene, *circHECTD1* diminished miRNA activity. As a result, *circHECTD1*-MIR142-*TIPARP* competition leads to modulation of astrocyte activation via autophagy in cerebral ischemia conditions.

In conclusion, we want to emphasize the significance of the studies by Han *et al.* [2018] and Bai *et al.* [2018] as important steps towards understanding the role of circRNAs in cerebral ischemia. It should be noted that a considerable number of recent studies show the complex nature of the regulation of pathogenesis and regeneration processes in ischemia, including the functioning of circRNAs. Han *et al.* [2018] and Bai *et al.* [2018] conclude that *circHECTD1* or *circDLGAP4* can serve as a novel biomarker and therapeutic target for stroke (1,25). We are optimistic about such conclusions because their work contributes to stimulating further research. Subsequent analysis of circRNA-miRNA-mRNA interactions is an important step in fully elucidating the mechanisms of damage and regeneration in cerebral ischemia and in determining strategies for achieving a neuroprotective effect in the conditions of this disease.

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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