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
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] 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

### 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.
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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