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Non-coding RNA and neuroinflammation: implications for the therapy of stroke

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ABSTRACT

Stroke is the major leading cause of death and serious, long-term disability with major economic consequences. At present, the lack of rapid diagnostic, prognostic biomarkers and effective treatment methods are two major challenges facing stroke. Circular RNAs (circRNAs) are potential clinical biomarkers in central nervous system diseases. However, the potential role of circRNAs in neuroinflammation and neuron functional recovery in acute ischaemic stroke (AIS) remains largely unknown. This review aimed to give an overview of the function of circRNAs in AIS and summarise the latest achievements in this field.

Stroke is a leading cause of death and long-term disability worldwide. Unfortunately, the lack of early diagnosis and prognostic biomarkers severely limits the diagnosis of stroke and is not conducive to the prognosis of patients. Currently, stroke therapies aim to establish early reperfusion through thrombolytic and/or mechanical recanalisation of obstructed blood vessels. Intravenous administration of recombinant tissue plasminogen activator (rt-PA) is the only Food and Drug Administration (FDA) approved therapy. Because of the narrow treatment time window and the side effects of rt-PA, the current treatment effect of stroke is not ideal. At present, several clinical trials have been carried out to identify the effective treatment of stroke, yet no effective pharmacological therapies have been found to protect damaged brain tissue after stroke.[1] In recent years, neuroprotective agents have attracted much attention, mainly through blocking one or more steps of neuronal ischaemic cascade to play a neuroprotective role. However, there is no ideal neuroprotective agent to improve clinical outcomes.[2 3] Therefore, a new treatment for stroke is urgently needed which can prolong the treatment time window of stroke and overcome the side effects of rt-PA.

Neuroinflammation is a major pathological event after ischaemic stroke, which can lead to secondary brain tissue injury and cause poor functional recovery. After the initial ischaemic injury, glial cells are activated and release neuroinflammatory factors. At the same time, the peripheral immune infiltration caused by the destruction of the blood-brain barrier (BBB) further enlarges the neuroinflammatory response and eventually leads to neuronal damage. Besides, many studies on glial cells and BBB also suggest the importance of these two factors in the regulation of stroke-induced neuroinflammatory response.[4]

BBB is a complex structure between cerebral vessels and brain tissues. It is the channel of material exchange between brain tissues and the surrounding environment, and plays an important role in maintaining the homeostasis of the central nervous system (CNS). BBB is mainly composed of microvascular endothelial cells and their tight junctions, basement membrane and pericyte, glial membrane formed by astrocyte end-foot and extracellular matrix, in which cerebrovascular endothelial cells are particularly important. Compared with peripheral blood vessels, microvascular endothelial cells in brain tissue overlap with each other. The tight connection between endothelial cells makes the permeability of BBB very poor and prevents harmful substances from entering the brain. With an ischaemic stroke, BBB dysfunction is characterised by structural damage and increased tight junction protein permeability. Peripheral chemicals, cells and body fluids extravasate into brain parenchyma across the impaired BBB, resulting in brain edema due to disrupted homeostasis of water and ion in the CNS. Peripheral inflammatory infiltration further aggravates inflammatory response and aggravates brain injury. While most consequences of BBB dysfunction are detrimental, one potential benefit is that it may enable therapeutic agents to reach brain targets. The repair of BBB is of great significance in inhibiting the CNS response and contributing to the recovery of stroke.[5]

Recent years, many studies have shown that astrocytes are involved in the pathological process of many nervous system diseases, such as stroke. Astrocytes are the most abundant glial cells in the CNS and play an important role in maintaining the structure and function of the brain. The physiological functions of astrocytes include structural support, formation of BBB, neuronal metabolism,

maintenance of extracellular environment stability, regulation of cerebral blood flow and stabilisation of intercellular communication. After stroke, astrocyte changes from resting state to activated state, and the proliferation and migration of activated astrocyte seriously damages brain tissue, leading to the formation of glial scar and affecting the repair of damaged brain tissue. Astrocyte plays an important role in supporting and regulating brain function, so it has both beneficial and adverse effects on the maintenance and repair of neurological function after stroke. Whether the activation and function of astrocytes should be weakened or strengthened after ischaemic stroke depends on the course of the lesion, the location of astrocytes and the specificity of cell subtypes. Therefore, it is necessary to investigate the molecular mechanism and signal pathway of astrocytes after stroke, so as to provide experimental basis for the treatment strategy of stroke.[6]

Circular RNA (circRNA) is one member of the non-coding RNA family, usually characterised by a stable structure and high tissue-specific expression.[7] circRNAs are highly expressed in the CNS and are involved in regulating physiological and pathophysiological processes. However, the potential role of circRNAs in acute ischaemic stroke (AIS) remains largely unknown. Our recent studies provide new insights into the function of circular RNA Hectd1 (circHectd1) and circular RNA DLGAP4 (circDLGAP4) in the diagnosis and treatment of cerebral ischaemia.

We used circRNA microarray to analyse the difference of circRNAs in the brain tissue between transient middle cerebral artery occlusion (tMCAO) model mice and normal mice. The results were validated with head-to-tail junction specific primers by PCR analysis. Noticeably, circHectd1 was highly expressed in the brain tissue of mice and dramatically increased after tMCAO. To further validate our results, 37 plasma samples from AIS patients were detected with 34 age and gender matched healthy controls, the levels of circHECTD1 were significantly increased in AIS patients, suggesting that the level of circHECTD1 is closely related to stroke. Further study reveals that knockdown of circHectd1 expression with circHectd1 small interfering RNA (siRNA) lentivirus could reduce brain infarction and improve neurological scores in tMCAO mice, evidenced by both 2,3,5-triphenyltetrazolium chloride strain assay and MRI. Besides, knock-down of circHECTD1 expression could inhibit astrocyte activation both in vivo and in vitro. We showed that circHECTD1 functions as a competing endogenous RNA that binds microRNA-142 (miR-142) to inhibit miR-142 activity, resulting in the inhibition of tetrachlorodibenzo-p-dioxin inducible poly (ADP-ribose) polymerase expression with subsequent inhibition of astrocyte activation via autophagy. In conclusion, our study shows that circHECTD1 is closely related to stroke. Targeting circHECTD1 can significantly improve the prognosis of stroke by inhibiting astrocyte activation and autophagy, suggesting that circHECTD1 may be a biomarker and therapeutic target for stroke.[8]

In addition to circHectd1, another article we published recently revealed the role of circDLGAP in stroke. We demonstrated that the levels of microRNA-143 (miR-143) were both increased in the plasma of tMCAO mice and AIS patients. MicroRNA-143 is a microRNA that can improve cerebrovascular injury. We further looked for the presence of some highly expressed circRNA in the brain which an act as competing endogenous RNAs (ceRNA) sponges and there's a binding site for mir-143, hence, we found circRNADLGAP4. CircDLGAP4 levels were significantly decreased in the plasma of tMCAO mice and AIS patients. Using a circDLGAP4 lentiviruses, the levels of circDLGAP4 was significantly overexpressed in tMCAO mice. Upregulation of circDLGAP4 expression significantly decreased infarct areas, attenuated neurological deficits and BBB injury induced by tMCAO. Taken together, circDLGAP4 plays an important role in stroke and BBB repair. It is a potential target for stroke treatment and has important clinical transformation significance.[9]

To our knowledge, we are the first to describe the efficacy of circRNA in an ischaemic stroke model. Here, we want to emphasise the significance of our investigation as an important step towards the translation of circRNA as a novel biomarker and therapeutic target for stroke.

It is extremely important to find objective and effective biomarkers for stroke, because these indicators can not only help the early diagnosis and prognosis assessment of stroke, but also serve as therapeutic targets to assist the development of new drugs. Universality, stroke can cause cascade changes of chemicals and transcripts in brain tissue. While conserved expression, defined specificity, high stability and abundance are the main characteristics of circRNAs that render them very attractive diagnostic tools for assessing diseases. Thus far, studies have demonstrated the potential of circRNA as a cancer diagnostic marker,[10] and recently studies have shown that circRNA is also a potential diagnostic marker for neurodegenerative diseases.[11] In the past few years, researchers have explored the distribution, changes, transcriptional profile and functions of circRNA under conditions of cerebral ischaemia. As early as 2016, in a mini review written by *Lu et al*[12], they put forward the idea of circRNA as potential clinical biomarkers for disorders in the CNS. They suggest that circRNA can regulate CNS function and many diseases, and can be used as a potential biomarker for the diagnosis and prognosis of CNS diseases, as well as combined with other biomarkers and imaging tools to improve the diagnostic power.[12] Subsequently, Mehta *et al* conducted a comprehensive circRNAs expression profile analysis on male tMCAO mice. MicroRNA binding sites, transcription factor binding and gene ontology of circRNAs altered after ischaemia were determined under cerebral ischaemia. In their study, a total of 1322 detectable circRNAs were comprehensively analysed, of which 283 had significant changes. Their research shows that these circRNAs altered after stroke may be controlled by a set of transcription factors. These circRNAs are involved in many processes and functions such as biological

regulation, metabolism, cell communication and binding with proteins, ions and nucleic acids.[13] Liu *et al* also studied the expression profile of circRNA in ischaemic stroke, confirmed that cricRNA is a potential target for diagnosis and treatment of stroke.[14]

To sum up, we want to emphasise the importance of our work as important steps towards understanding the role of circRNAs in cerebral ischaemia. We are optimistic about that circRNA can act as a diagnostic marker and therapeutic target for stroke.

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