

# Advances in the diagnosis of exosomal miRNAs in ischemic stroke

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**Abstract:** Early diagnosis, early treatment, and improved prognosis in patients with ischemic stroke are vital requirements. Current clinical practices for the diagnosis of stroke include computed tomography, magnetic resonance imaging, and other traditional imaging methods to quickly check the location, volume, etc, in the hospital; however, diagnosis of the underlying cause of infarction is not effective with these practices. Owing to the coexistence of various etiologies, accurate and timely diagnosis using routine hematology and biochemical tests remains a challenge. Exosomes are membrane vesicles, approximately 30–150 nm in diameter, which fuse with cell membrane and are released into the extracellular space. As one of the research hotspots in the field of medicine in recent years, exosomes can participate in immune response, antigen presentation, cell migration, tumor invasion, and so on. Owing to the important role played by the miRNAs contained in exosomes, the latter have shown great potential in the diagnosis and treatment of ischemic stroke. This article reviews the progress made regarding the exosomal miRNAs as ischemic stroke biomarkers.

**Keywords:** exosome, transporter, prognosis, intercellular communication

## Introduction

Stroke is one of the leading causes of disability in both developing and developed countries. Oxygen and nutrient deficiencies in the brain tissue, due to reduced supply of cerebral blood flow, eventually lead to neuronal cell death and cerebral infarction. Stroke has the characteristics of high incidence, high recurrence rate, high disability rate, multiple complications, and high mortality. According to the 2018 China Stroke Prevention Report,<sup>1</sup> the standard incidence rate of first stroke in 40–74-year-olds has increased by 8.3% per year. The standard rate of stroke in residents aged 40 years or older has increased from 1.89% in 2012 to 2.19% in 2016. In China, approximately 75% of the patients with stroke have ischemic stroke (IS).<sup>2</sup> Currently, stroke diagnosis relies on magnetic resonance imaging and computed tomography. However, such expensive machines have associated limitations. When symptoms are mild or difficult to distinguish from other neurological and non-neurological diseases, it is often difficult for doctors to make objective judgment based on image results. At present, a variety of related biomarkers have been found to be involved in oxidative damage, inflammatory reactions, and brain damage, although all these markers have certain limitations in clinical applications.<sup>3</sup> Therefore, one of the challenges in IS-related research is to find more effective and reliable biomarkers.

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## Exosomes: overview

Exosomes are essentially composed of a lipid bilayer that can be detected in most body fluids such as peripheral blood, saliva, and ascites. Initially, exosomes were thought to be a way of waste excretion by cells. Valadi et al (2007)<sup>4</sup> first identified the presence of a new vector for mRNA and miRNA for intercellular genetic material exchange and communication. Exosomes form an important means of intercellular substance transfer and information exchange, and play biological functions in immune response, besides RNA and protein transport. Studies have shown that exosomes also have the ability to cross the blood–brain barrier and quite specifically execute long-distance propagation in body fluids under different conditions.<sup>5,6</sup> In recent years, studies have suggested exosomal miRNAs to possibly be novel biomarkers and therapeutic targets for various diseases, such as tumors, neurodegenerative diseases, and vascular diseases.<sup>7–9</sup>

## Exosomal communication between cells

Coordination of intercellular communication plays an important role in the regulation of homeostasis in the body. Intercellular communication can be roughly divided into direct contact and indirect contact, the mechanism including cell adhesion, gap junction, and release of biologically active molecules.<sup>10</sup> Studies have shown the transportation of bioactive molecules by extracellular vesicles (EVs) to be a common mechanism for intercellular communication. Exosomes, like EVs, transport RNA (including mRNA and miRNA) and other non-coding RNAs.<sup>4</sup> They were later discovered to carry single- and double-stranded DNA, amplified oncogene sequences, transposable elements, as well as some proteins involved in exosomes biogenesis, exhibiting tissue/cell type specificity.<sup>11–13</sup> Studies have also shown exosomes to contain a variety of proteins involved in brain repair, including synaptic transmission and axon growth.<sup>14,15</sup> They are not only transport carriers between cells but also regulate the transmission of information molecules, with targeted regulation.<sup>16</sup> An advantage of exosomes being intercellular communication media is their delivery of information molecules to multiple recipient cells. Exosomal miRNAs alter gene expression and regulate cell function in recipient cells through the transfer of exosomes. miRNA is a non-coding single-stranded RNA of approximately 22 nucleotides that acts as a signaling molecule to convey genetic information

and regulates cellular functions without direct contact.<sup>4</sup> Therefore, this new intercellular communication mechanism, mediated by exosomes, has gradually attracted widespread attention.

## Exosomal miRNAs as an early diagnostic and prognostic biomarker for IS

Biomarkers are indicators that can objectively detect and evaluate normal physiological and pathological processes or drug responses. Blood cells and endothelial cells release exosomes into the blood in response to IS. CD9, Alix, CD63, TSG101, and CD81 are currently believed to be marker proteins of exosomes.<sup>4,13</sup> Chen et al<sup>17</sup> had shown a possibility of exosomal miR-223 to be associated with acute ischemic stroke (AIS), severity, and short-term prognosis. Zhou et al<sup>18</sup> had suggested that exosomal miR-134 can be used as a novel biomarker for diagnosis and prognosis of stroke. Besides, it is strongly positively correlated with the expression of IL-6 and hs-CRP. Ji et al<sup>19</sup> had predicted serum exosomal miR-9 and miR-124 to possibly be biomarkers for assessing the extent of injury caused by ischemic injury; however, further research would be required to explore their potential role after being released from other brain tissues in post-stroke complications.

IS is divided into different stages according to the onset time. Following IS, many proteins, nucleic acids, or metabolites are produced, which may be related to the specific physiological and pathological processes of IS. However, their specificity and ability to distinguish different the stages of stroke remain uncertain.<sup>20</sup> Early and accurate diagnosis can greatly improve the prognosis of patients. Wang et al<sup>21</sup> further subdivided the disease groups into hyperacute ischemic stroke (HIS) group, AIS group, subacute ischemic stroke (SIS) group, and restorative ischemic stroke (RIS) group. The levels of exosomal miR-21-5p in the SIS and RIS groups were significantly higher than those in the control group. The level of miR-30a-5p was significantly enhanced in the HIS group, whereas it was reduced in the AIS group. Both AIS and HIS groups showed lower miRNAs. The combination of exosomal miR-21-5p and miRNA-30a-5p is suggested to be a promising biomarker for diagnosing IS, and distinguishing between HIS, SIS, and RIS, especially miRNA-30a-5p for the diagnosis of HIS. Li et al<sup>22</sup> found the expression levels of exosomal miR-422a and miR-125b-2-3p in the SIS group to be significantly lowered, and that

of miR-422a in the AIS group to be elevated. In addition, expression levels of SIS exosomal miR-422a and miR-125b-2-3p were significantly lower than those in the acute phase, which may have clinical value in thrombolytic therapy in ischemic cerebral infarction.

Although transient ischemic attack (TIA) can be diagnosed during windowing, new imaging techniques are complex and expensive. Li et al<sup>23</sup> established 5-min, 10-min, and 2-hr models of cerebral artery occlusion. Plasma exosomal miRNA-122-5p was found to be significantly downregulated in a 10-min ischemic rat model, whereas plasma exosomal miRNA-300-3p was significantly upregulated in a 5-min ischemic rat model. Therefore, plasma exosomal miRNA-122-5p and miRNA-300-3p may be suggested as blood-based TIA biomarkers.

## Association and difference between circulating miRNAs and exosomal miRNAs as biomarkers

Circulating miRNAs are often used as non-invasive diagnostic and prognostic biomarkers for diseases since they are stable and easy to detect in blood samples. Vijayan et al<sup>24</sup> had considered the circulating levels of PC-3p-57664, PC-5p-12969, and miR-211-5p to be potential biomarkers for the diagnosis of IS. The same group<sup>25</sup> found PC-5P-12969 to not only be a potential candidate for IS peripheral markers but also be a drug target for IS. This study confirmed, for the first time, that miRNA PC-5P-12969 is an IS biomarker. Interestingly, both circulating miRNAs and exosomal miRNAs are new potential serum markers for IS. Although circulating miRNAs contain exosomal miRNAs, the two may not necessarily be correlated. Chen et al<sup>26</sup> studied the changes in exosomal miR-126 levels, which may be more sensitive to cerebral ischemia, whereas serum miR-126 may be more specific to the severity of ischemia. However, since no correlation between serum and exosomal miR-126 levels could be established in this study, further studies were recommended to determine how the signals are classified between compartments. miRNAs may be stable in circulation, which contributes to their being disease biomarkers.<sup>27</sup> To some extent, this stability is thought to be mediated via exosomes, possibly due to encapsulation and as delivery systems that protect miRNAs from external RNases. Some studies support the idea of exosome-mediated functional miRNA transfer.<sup>4,28–30</sup> Although both circulating miRNAs and exosomal miRNAs can serve as serum markers for IS, there are different mechanisms for the expression of both the two,

during the same disease process. miRNAs are currently known to be involved in central nervous system function and disease,<sup>31</sup> further research is warranted to explore the mechanism underlying the involvement of exosomal and total serum microRNA in stroke pathophysiology.

## Other types of non-coding RNAs in IS

A recent study<sup>32</sup> had analyzed plasma exosomal RNA sequences. Sequence analysis showed miRNAs to be the most abundant RNA species, accounting for 76.20% of the total. Other important RNA species included ribosomal RNA (9.16%), long non-coding RNA (3.36%), and Piwi-interacting RNA (1.31%). Although RNA biomolecules are continuously released from cells into body fluids, they have a very short half-life due to the rapid degradation by ubiquitous RNases and other chemical processes. Patel et al<sup>33</sup> found that the long non-coding RNA MALAT1, in adipose-derived stem cell exosomes, has enormous therapeutic potential for the treating traumatic brain injury.

In recent years, some other non-coding RNAs have also been implicated in ischemic stroke. Zhu et al<sup>34</sup> found lncRNA MIAT to be applicable as a potential marker for IS. lncRNA H19 promotes neuroinflammation by driving HDAC1-dependent M1 microglial polarization, thus suggesting the applicability of lncRNA H19 in the diagnosis and treatment of ischemic stroke.<sup>35</sup> CircDLGAP4, originally identified as a sponge of miR-143 in ischemic stroke outcome, may serve as a novel therapeutic target for acute ischemic injury.<sup>36</sup> Han et al<sup>37</sup> found a correlation of circHCTD1 and its coupling mechanism with cerebral ischemia, which can, in turn, be used as a novel biomarker and therapeutic target for stroke.

## Summary and outlook

Exosomes show great potential as biomarkers, drug carriers, in neurovascular remodeling, and in treatment, owing to their unique advantages of crossing the blood–brain barrier. However, it still faces the following challenges: (1) Although exosomes mediate intercellular communication via a new mechanism, the specific details of signal transduction are still unclear; (2) the mechanism of distribution of the exosomal and triggering release in different cells also remains to be explored; (3) most of the research on exosomes is currently limited to miRNA; other specific RNAs are less found; (4) while separation and purification of exosomes are particularly important, no stable and efficient extraction and

preparation techniques are currently known; (5) stability of the test results, including fewer samples, more limited sources, and more confounding factors in blood samples, needs to be improved. Taken together, a follow-up study is required to further expand our sample verification. The study of exosomes for the diagnosis and prognosis of IS still has a long way to go; future research can pave the way for clinical application of exosomes in the field of medicine.

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## Disclosure

The authors report no conflicts of interest in this work.

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