Exosomal Noncoding RNAs in Ischemic Stroke

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Ischemic stroke is a life-threatening condition that also frequently results in long-term disability. Exosomes and exosomal noncoding RNAs have been found to be involved in the pathophysiological progression of ischemic stroke, including atherosclerosis, apoptosis, inflammation, oxidative stress, and neurovascular remodeling.

exosomes circRNA ischemic stroke

1. The Potential Diagnostic Value of Exosomal Noncoding RNAs in Ischemic Stroke

An array of evidence has revealed that exosomes may be ideal biomarkers for different diseases, including ischemic stroke, due to their convenient acquisition and widespread distribution in diverse biological fluids. Previous studies have detected that various noncoding RNAs are differentially expressed under hypoxic and hypovolemic states in animal models and patients with ischemic stroke and they could be used as monitoring factors in the progression of ischemic stroke [1][2]. The following contents will describe the altered expression profile of exosomal noncoding RNAs in the disparate models of ischemic stroke (**Table 1**).

Table 1. Differential expression profiling of exosomal noncoding RNAs in ischemic stroke.

Models and Tissues	Detection Method	Changes of Expression Profiles	Focal ncRNAs	Reference
Exosomes from peripheral serum in stroke patients	RNA-Seq and qRT-PCR	1096 lncRNAs covering 307 showed elevated expression while 789 showed decline	lnc-CRKL-2 and lnc-NTRK3-4 ↑ RPS6KA2-AS1 and lnc-CALM1-7 ↓	Xu [3]
Exosomes from peripheral plasma in stroke patients	HTS and qRT-PCR	1020 lncRNAs were differentially expressed, 226 lncRNAs increased and	Most: novel lnc_000288 ↑; novel lnc_000285 ↓ lnc_002015/hsa-mir342/PTPRC/AAMP; lnc001350/hsa-mir-3127/ST14	Zhang [4]

Models and Tissues	Detection Method	Changes of Expression Profiles	Focal ncRNAs	Reference
		794 lncRNAs decreased levels		
Exosomes from peripheral plasma in stroke patients	RNA-Seq and qRT-PCR	319 lncRNAs including 97 increased expression and 222 showed reduction	lnc_000048, lnc_001350 and lnc_016442 ↑ lnc_002015 ↓	Zhang [5]
Exosomes from peripheral plasma in stroke patients	HTS	3540 circRNAs, 1177 increased expression and 2363 decrease expression	/	Xu [6]
Exosomes from peripheral plasma in stroke patients	RNA-Seq and qRT-PCR	25 circRNAs, 9 circRNAs were significantly upregulated and 16 circRNAs were significantly downregulated	hsa_circ_0000698/hsa_circ_0002775/hsa_circ_0005585/hsa_circ_0043837 ↓; hsa-miR-16 ↑; VWF ↓; novel_circ_0010155 ↓; hsa-miR-939 ↑; septin 9 and MYLK2 ↓	Xiao [7]
Exosomes from peripheral plasma in stroke patients	RNA-Seq and qRT-PCR	26 circRNAs, 7 circRNAs were significantly upregulated and 19 circRNAs were significantly downregulated	circ_0043837 circ_0001801	Xiao [8]

↑ upregulation; ↓ downregulation.

The team of Xu reported the differential expression of exosomal long noncoding RNAs (lncRNAs) in acute minor stroke patients whose NIHSS (National Institute of Health stroke scale) scores were no more than 4 compared with healthy controls. One hundred patients with symptoms appearing within 24 h, paired with 100 controls, were enrolled in their study and blood samples were obtained without any treatment. In their RNA sequencing (RNA-seq) analysis, containing three-pair subjects, 1096 exosomal lncRNAs had significantly altered expression levels (>1.5-fold, $p < 0.05$), including 307 with increased expression and 789 with decreased expression. After this, they selected the neurotrophin signaling pathway and four relevant lncRNAs (upregulated: lnc-CRKL-2, lnc-NTRK3-4; downregulated: RPS6KA2-AS1, lnc-CALM1-7) according to GO (Gene Ontology) enrichment analysis and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis for next-step validation. The results of RT-qPCR (quantitative real-time polymerase chain reaction) testing of these four lncRNAs showed consistent trends with their preceding experiment between exosomes from patients and controls [3]. Their research illustrated that

exosomal lncRNAs may be a viable option for the early detection of the disease, but further exploration of the molecular bases and underlying signal pathways is still needed.

Soon after these results were presented, Zhang et al. [4] performed a comprehensive exosomal lncRNA sequencing with related networks in order to explore their diagnostic potential and underlying mechanisms in large-artery atherosclerotic (LAA) stroke. Thirty-five LAA patients (>50% extracranial or intracranial arteries stenosis) within 72 h of stroke onset and an equal number of controls were enrolled in their study, and all subjects were matched by clinical characteristics, including age, sex, and vascular risk factors. Initially, they found that 1060 lncRNAs were differentially expressed, covering 226 lncRNAs that were upregulated and 794 that were downregulated significantly in the LAA cases versus controls using the high-throughput sequencing (HTS) method (5 paired samples). The chromosomal distribution of differentially expressed genes showed differentiated transcripts spread over every chromosome and chromosome 1 contained the most. Based on the target gene functions and differential expression, 8 hub lncRNAs relevant to atherosclerosis were selected in order to construct related networks according to their reads and changed folds, and biological function analysis of these networks by GO and KEGG pathways showed that inflammation, immunity, cell adhesion, and apoptosis may be attributed to the progression of atherosclerosis. After this analysis, the authors validated the observed differential expression from the HTS results of 21 hub genes involved in the lncRNA-related networks by a quantitative real-time PCR (qRT-PCR) experiment in a cohort of stroke patients (30 stroke versus 30 control). In the validation set, they constructed an exo-lncRNA-related diagnostic model of LAA stroke and focused on two lncRNAs, lnc_001350 and lnc_002015, as propelling and defensive factors for the occurrence of LAA stroke ($p < 0.05$). Furthermore, the diagnostic efficacy of exo-lncRNA-related networks could be higher than any single gene in the network as the area under the curve (AUC) of the lnc_002015-related network and lnc_001350-related network were 0.959 and 0.970, respectively. They also presented results on the lnc_002015/hsa-miR-342/PTPRC/AAMP and lnc001350/hsa-miR-3127/ST14 pathways since PTPRC and miR-342 have previously been reported to mediate the progression of AS and the inflammatory response. Of note is that the two lncRNAs in question are more probably located in the cytoplasm, which would enhance the reliability of exo-lncRNA-related functional networks.

Recently, Zhang's team affirmed the diagnostic and prognostic value of exosomal lncRNAs for LAA stroke in the clinical setting; furthermore, their research indicated that exosomal lncRNAs rather than plasmatic lncRNAs might be particular biomarkers for LAA stroke, with an obvious advantage in the AUC. They randomly assigned 602 participants to a discovery set (n = 12), validation set (n = 80), and replication set (n = 510), and RNA sequencing and qRT-PCR were used to detect the differential expression of lncRNAs. Firstly, 222 downregulated and 97 upregulated lncRNAs were discerned in the discovery set ($|\log_2 \text{fold change}| \geq 2$, $p < 0.05$), and functional enrichment analysis showed that the target genes of these exo-lncRNAs were mainly involved in the AS-related pathological process. After validation testing and subgroup analysis, the results indicated that upregulated exo-lnc_000048, exo-lnc_001350, and exo-lnc_016442 were consistent with an increased risk of LAA stroke. The AUC of the combined three exo-lncRNAs was 0.936, which was not influenced by the traditional biomarkers of IS (triglycerides, total cholesterol, low-density lipoprotein). Moreover, the adjusted logistic analysis revealed that the combination of exo-lnc_001350, exo-lnc_016442, and NIHSS provided better prognostic capacity for one-month unfavorable outcomes, independent of sex, age, and the above traditional biomarkers [5]. Overall, these results

provided exosomal lncRNA models with satisfactory diagnostic and prognostic valuew in LAA stroke. Other multicenter and large-scale studies are warranted to verify these results, which could be meaningful for the rapid detection and preferable functional outcomes of the disease.

According to their research, it can be recognized the diagnostic value of plasma exosomal lncRNAs and related networks in acute minor stroke as well as LAA stroke; moreover, the diagnostic possibilities of exosomal RNAs in the different types of ischemic stroke are worth consideration.

3. The Expression Profile of Exosomal Circular RNAs

Xu et al. discovered altered plasma circular RNA (circRNA) expression profiles in IS patients within 72 h of onset compared to healthy controls. They found a total of 3540 differentially expressed circRNAs in exosomes, including 1177 with increased expressions and 2363 with decreased expressions (fold change > 2, $p < 0.05$). Among the entirety of the abnormally expressed circRNAs, most of them were located at chromosome 2 and were less than 500 nucleotides in length; in addition, exons and introns made up over 60% of the source regions. Bioinformatics analysis revealed and predicted underlying pathways in the IS pathology, such as the MAPK signaling pathway, lipids, and atherosclerosis, neurotrophin signaling pathway, etc. Moreover, ten hub genes were selected from the protein_protein interaction network, providing clues for the subsequent mechanistic research of noncoding RNAs in ischemic stroke [6]. It should be pointed out that the NIHSS scores of patients in their study were 11–25, while mild stroke was not included; more research covering different subtypes and orders of severity for stroke is needed in the future.

In a study by Xiao et al. [7], a comprehensive examination of exosomal circRNAs isolated from the peripheral blood of LAA stroke patients was performed. There were 37 patients within 72 h of symptom onset paired with 37 normal controls in their study, and stenosis of 50% or above in intracranial or extracranial arteries was discerned in all cases. At first, the authors randomly selected five paired LAA patients and controls on whom to conduct RNA-Seq analysis and identified 25 differentially expressed exosomal circRNAs among a pool of 462 exosomal circRNAs in total. The circRNAs were primarily from gene exons, and their lengths were mostly less than 2000 bp. In the LAA group, the expression of nine circRNAs in the exosomes was significantly increased, and sixteen circRNAs were significantly decreased (≥1.5-fold, $p < 0.05$). Subsequent GO and KEGG enrichment analysis showed that these differentially expressed exosomal circRNAs may be involved in the inflammatory response of LAA stroke, so the authors also constructed circRNA_miRNA_mRNA ceRNA (competing endogenous RNA) networks and selected two of these for validation using qRT-PCR in both LAA subjects and controls (32 versus 32). Here, they observed largely similar trends in RNA-seq as well as the significant differential expression of RNAs (circRNAs, miRNAs, and mRNAs) ($p < 0.05$). Translatable analysis showed that exosomal circRNAs contained internal ribosome entry sites, junction sequences, and open reading frames, indicating the translation potential of exosomal circRNAs. Finally, they predicted the diagnostic value of circRNAs in their validation phase by receiver operating characteristic (ROC) curve analysis and identified two circRNAs (novel_circ_001015, hsa_circ_0005585) as possible novel biomarkers for LAA stroke, and the AUC of combined circRNAs increased compared with individual ones. Taken together, these results provide information on exosomal circRNA ceRNA networks and potential encoding proteins in the

progression of LAA stroke, and also showcase the possible diagnostic efficacy and biological functions of exosomal circRNA. Given that their research was aimed at mild-to-moderate LAA stroke patients (basal NIHSS score: 4.78 ± 1.00), the associations between exosomal circRNAs and different severity and subtypes of ischemic stroke warrant further study in the future.

Next, their team expanded the sample size and continued to explore the diagnostic and predictive value of exosomal circRNAs for LAA stroke. A total of 196 and 149 participants with LAA stroke and normal controls were enrolled, respectively. In the discovery experiment, they identified 26 differentially expressed circRNAs in LAA subjects compared to normal controls (|fold change| ≥ 1.5, $p < 0.05$), including 7 with significantly elevated and 19 with significantly decreased expression. In addition to the validation and replication stage, they constructed a logistic regressive model and found that circ_0043837 and circ_0001801 remained powerful and independent factors; the combined AUC of these two circRNAs was 0.825, and the diagnostic efficacy of exsomal circRNA was better than circRNA in the plasma. Furthermore, they found that exosomal circ_0043837 and circ_0001801 were potential biomarkers for predicting atherosclerotic plaque rupture; similarly, the exosomes showed superior diagnostic efficacy to plasma. The nomogram also revealed the potential predictive value of exosomal circRNAs in plaque instability. Besides this, they suggested that these two exo-circRNAs exerted a protective role in ischemic stroke, as a negative correlation between their expression and the NIHSS scores was found [8]. Considering that this was a single-center study, future multicenter studies are needed to validate these results and avoid selective bias.

All of the studies highlighted in above contents showed that exosomal long noncoding RNAs and circular RNAs in the blood of ischemic stroke patients display an altered expression profile, indicating that exosomal noncoding RNAs have the potential to become both diagnostic and prognostic biomarkers in the occurrence and development of ischemic stroke.

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