Published in final edited form as:

J Stroke Cerebrovasc Dis. 2019 January ; 28(1): 121–124. doi:10.1016/j.jstrokecerebrovasdis.2018.09.018.

Differential MicroRNA Expression in Cardioembolic Stroke

Janhavi M. Modak, MD[1], **Meaghan Roy-O'Reilly, MS**[2], **Liang Zhu, PhD**[2], **Ilene Staff, PhD**[1], and **Louise D. McCullough, MD/PhD**[2]

[1]Department of Neurology, Hartford Hospital, 80 Seymour Street, Hartford CT 06106

[2]Department of Neurology, University of Texas Health Science Center, Houston TX 77030

Abstract

Background: MicroRNAs (miRNA) are a class of small, endogenous (17– 25 nucleotide) non-coding ribonucleic acids implicated in the transcriptional and post-transcriptional regulation of gene expression. This study examines stroke specific miRNA expression in large vessel territory cardioembolic stroke.

Methods: Peripheral blood was collected from controls and ischemic stroke patients 24 hours after stroke onset. Whole blood miRNA was isolated and analyzed for differential expression. A total of 16 patients with acute middle cerebral artery (MCA) territory strokes of cardioembolic origin were included in this pilot study. MiRNA profiling was conducted by miRCURY LNA™ microRNA Array.

Results: In patients with cardioembolic stroke, significant differential expression of 14 miRNAs was observed when compared to controls. Ten of these microRNA had not previously been associated with ischemic stroke (miR-664a-3p, −2116–5pp, −4531, −4765–5p, −647, −4709–3p, −4742–3p, −5584–3p, −4756–3p, −5187–3p). Sub-analysis of severe strokes (NIHSS > 10) identified an additional 5 differentially expressed miRNA. No significant effects of sex or tPA treatment were seen on miRNA expression.

Conclusions: Ischemic stroke patients show a differential miRNA expression profile as compared to controls. These new associations between circulating miRNAs and ischemic stroke may help to refine stroke subtype diagnosis and identify novel therapeutic miRNA targets for the treatment of ischemic stroke.

Keywords

Acute ischemic stroke; acute stroke; blood biomarkers; cardioembolic stroke; microRNA; translational research; clinical research; clinical stroke

Address Correspondence to: Louise D. McCullough, Department of Neurology, University of Texas Health Science Center, Houston, 77030. Louise.D.McCullough@uth.tmc.edu. Telephone: 1-713-500-7065. Fax: 713-500-0660.

Disclosures: None.

Introduction:

Stroke is a leading cause of disability and death worldwide [1]. Prior studies have identified numerous potential biomarkers for ischemic stroke, including RNA-based biomarkers [1]. As RNAs are continuously transcribed, translated and turned over in response to physiologic and pathologic stimuli, the RNA profile of the cell serves as a useful reflection of its current functional state [1–3].

MicroRNAs (miRNA) are a class of endogenous, small (~17–25 nucleotide), non-coding ribonucleic acids implicated in the transcriptional and post-transcriptional regulation of gene expression [3]. MiRNA can impact cellular function by suppressing or activating downstream mRNA targets, which in turn regulates protein expression [2–3]. Pre-clinical studies have demonstrated specific changes in miRNA expression profiles after ischemic stroke [3–5]. In addition, human studies have identified specific miRNA expression profiles associated with ischemic stroke, including those involved in thrombosis and leukocyte extravasation [4–8]. However, at this point, there are no established serum biomarkers in routine clinical practice that predict stroke risk, etiology or outcome. In this study, we sought to discover novel stroke-associated miRNA by analyzing a highly homogenous subset of patients with large cerebral artery strokes of embolic origin.

Materials and Methods:

Ischemic stroke patients admitted to a tertiary hospital between January 2011 and March 2014 were considered for this pilot study. Blood samples were collected at 24 ± 6 hours from symptom onset. Ethical approval for human studies and waiver of written informed consent was obtained from the Institutional Review Board at Hartford Hospital and the University of Connecticut Health Center. All procedures for specimen collection and analysis were conducted in accordance with institutional guidelines.

Inclusion/Exclusion criteria:

Patients above 18 years of age presenting with an acute ischemic stroke were considered for study inclusion. Stroke diagnosis was confirmed by clinical and radiologic evaluation. Among ischemic stroke patients, only those with cardioembolic strokes in the middle cerebral artery (MCA) territory were considered for inclusion (n=16). Exclusion criteria included history of brain neoplasia, active peripheral malignancy, past traumatic brain injury or brain hemorrhage or strokes not secondary to a cardioembolic source. Blood collected from outpatients with no known acute/chronic neurological deficits and matched vascular risk factors served as controls (n=8).

MicroRNA isolation:

Blood samples were collected in PAX-gene tubes. miRNA profiling and analysis was performed by Exiqon. The quality of RNA was assessed using an Agilent 2100 bio-analyzer profile. Total RNA was labeled using the miRCURY LNA™ microRNA Hi-Power Labeling Kit, Hy3™/Hy5™ prior to analysis by miRCURY LNA™ microRNA Array 7th Gen (Exiqon) with capture probes targeting all microRNAs for human, mouse or rat registered in the miRBASE 18.0. Hybridization was performed using a Tecan HS4800™ hybridization

station (Tecan). After hybridization, microarray slides were scanned using the Agilent G2565BA Microarray Scanner System (Agilent Technologies, Inc.) and image analysis was performed using ImaGeneR 9 (Denmark).

Statistics:

A total of 560 microRNAs were analyzed using the described micro-array technique. Based on our literature review, 173 miRNAs were analyzed for differential expression between ischemic stroke cases and controls. For expression analysis, calculated p-values were based on Wilcoxon rank sum test. The Benjamini and Hochberg multiple testing adjustment method was applied to control false discovery rate at 0.05. Statistical analysis was performed using SAS 9.4 (Cary, NC).

Results:

The demographic characteristics of study patients are given in Table 1. Amongst all stroke cases, 50% patients had a left MCA infarct, and 50% had a right MCA stroke. The mean NIH stroke scale on admission was 12, with the NIHSS ranging from 5 to 19. The stroke etiology was cardioembolic in 14 cases (87.5% patients) whereas 2 cases (12.5%) had a high likelihood of a cardioembolic etiology based on minimal intracranial or extracranial atherosclerosis. Amongst patients with confirmed cardioembolic strokes, 71.4% had atrial fibrillation and 28.6% cases were not related to Afib (one patient had a mechanical aortic valve and had not been on anticoagulation, one had a low ejection fraction, and another was noted to have a left ventricular thrombus post myocardial infarction).

Our analysis identified significant differential expression of 14 miRNAs (11 downregulated, 3 upregulated) in acute ischemic stroke patients as compared to controls. MicroRNAs miR-1273e (log FC −0.426, p=0.011), miR-5187–3p (log FC −0.426, p=0.011) were found to be downregulated in stroke patients. Other miRNAs showing significant downregulation included let 7e-5p (log FC −0.309, p=0.039); miR-4709–3p (log FC −0.372, p=0.023), miR-4756–3p (log FC −0.372, p=0.023), miR-5584–3p (log FC −0.312, p=0.024), miR-647 (log FC −0.283, p=0.024). MicroRNAs miR-4742–3p (log FC −0.289, p=0.038), miR-4764–5p (log FC −0.251, p=0.041), miR-4531(log FC −0.194, p=0.042) and miR-2116–5p (log FC −0.179, p=0.043) were also depressed in stroke patients compared to controls. MicroRNAs miR-664a-3p (log FC 0.227, p=0.024), miR-943 (log FC 0.234, p=0.043) and miR-145–5p (log FC 0.399 p=0.039) were significantly upregulated in patients with acute ischemic stroke (Table 2). Interestingly, 10 of our identified miRNA have not been previously described in association with acute ischemic stroke.

As initial stroke severity is a strong predictor of stroke pathophysiology and outcome, we then conducted a subgroup analysis comparing acute ischemic stroke patients with more severe stroke deficits with an initial presenting NIH Stroke Scale (NIHSS) above 10 (n=10) with controls (n=8). Additional miRNAs, including miR-29a-5p (p=0.022), miR-151a-3p (p=0.023), miR-487b-3p (p=0.025) and let-7b-3p (p=0.046), were significantly upregulated in severe ischemic stroke cases (Table 3). MicroRNAs miR-4531 (p=0.023) and miR-15b-5p (p=0.048) were significantly downregulated in these patients. Importantly, no significant sex

differences or thrombolysis treatment effects on miRNA expression were observed although this is likely due to the low number of patient samples.

Discussion:

MicroRNAs are key regulators of gene function and play a pivotal role in the modulation of the complex cascade of molecular signaling associated with neuronal injury. Specific miRNAs have been associated with ischemic stroke and related processes, including atherosclerosis and inflammation [3,4]. Current evidence indicates that miRNAs may act as stroke biomarkers as well potential targets for therapy [4–8]. While the majority of miRNAs are intracellular, miRNA also exists extracellularly. The circulating miRNA identified in our study of whole blood may have been derived from plasma and/or peripheral blood cells [8]. Further investigation of these miRNA may reveal new mechanisms of regulation in response to cerebral injury.

Our study identified 10 miRNA that have not previously been associated with ischemic stroke (Table 2) [3–8]. MicroRNAs miR-4531, miR-4756–3p and miR-5584–3p were downregulated in patients with ischemic stroke. Prior studies have not identified these microRNAs in patients with stroke/cerebrovascular disease or other neurologic or medical comorbidities. In addition, we identified differential regulation in microRNAs including miR 5187–3p, miR 4742–3p, miR-664a-3p, miR-647, miR-4764–5p, miR-2116–5p and miR-943 which were not previously described in the context of cerebral ischemia.

Several of these miRNA have been previously described in the context of other diseases. Transforming growth factor beta receptor II (TGFBR II), a target of miR-664, plays a critical role in the TGF beta signaling pathway that is necessary for tissue repair [9]. The upregulation of miR-664 in stroke patients seen in our study could contribute to increased TGF beta signaling, resulting in cellular proliferation and angiogenesis that may impact post-stroke recovery.

In our study, miR-4742 and miR-4709–3p were downregulated in stroke patients. Both of these miRNA have previously identified roles in the phosphotidyl inositol-3-kinase (PI3K-Akt) signaling pathway [10]. The PI3K pathway regulates neural stem cell physiology, and is thus instrumental in cell survival or regeneration after cerebral infarction. Changes in the level of these miRNA may drive the alterations in PI3K pathway signaling seen after ischemic stroke. Additionally, we found down-regulation of miR-647 and miR-2116 in stroke patients. Targets of these microRNAs have been linked to the regulation of immune and metabolic pathways, but no studies to date have found an association between these microRNA and ischemic stroke [11,12].

In addition to the novel ischemic stroke miRNA, our study confirmed differential expression of microRNAs miR-1273e, miR-15b-5p and miR-145, which have all been previously associated with ischemic stroke and atherosclerosis [3,4,13]. We also noted differential expression of hsa-let-7e-5p, hsa-let-7b, miR-487b-3p which have been previously described in cases of ischemic stroke. Subsequent subset analysis found no significant effect of patient

sex or tPA treatment on miRNA expression after ischemic stroke, likely due to the small sample size.

Summary:

This study identified several new miRNAs in specifically selected patients with cardioembolic strokes of similar stroke location and infarct size. Our selection methods reduced variability but did lead to a small sample size. This study should serve as a pilot study to evaluate microRNA expression in patients with a MCA territory cardioembolic stroke. Our work has identified multiple novel microRNAs that have not been previously described in the context of ischemic stroke, due in part to our focus on this homogeneous subset of ischemic stroke patients (middle cerebral artery strokes of cardioembolic origin). These newly identified microRNAs may provide a reliable signature for cryptogenic strokes of possible cardioembolic origin but this will need to be validated in larger cohorts. Furthermore, identification of these miRNAs may allow for the development of new molecular targets and therapeutic strategies for management of large hemispheric embolic strokes.

Acknowledgments

Funded by NS094543 and NS076293 (to Louise D McCullough) and F30 NS098628 (To Meaghan Roy O'Reilly).

References:

1. Whiteley W, Tseng MC, Sandercock P: Blood biomarkers in the diagnosis of ischemic stroke: a systematic review. Stroke. 2008;39:2902–9. [PubMed: 18658039]
2. Rawlings-Goss RA, Campbell MC, Tishkoff SA: Global population-specific variation in miRNA associated with cancer risk and clinical biomarkers. BMC Med Genomics 2014;7:53. [PubMed: 25169894]
3. Rink C, Khanna S: MicroRNA in ischemic stroke etiology and pathology. Physiol Genomics. 2011;43:521–8. [PubMed: 20841499]
4. Koutsis G, Siasos G, Spengos K: The emerging role of microRNA in stroke. Curr Top Med Chem 2013;13:1573–88. [PubMed: 23745809]
5. Sepramaniam S, Tan JR, Tan KS, et al.: Circulating microRNAs as biomarkers of acute stroke. Int J Mol Sci 2014;15:1418–32. [PubMed: 24447930]
6. Jickling GC, Ander BP, Zhan X, et al.: microRNA expression in peripheral blood cells following acute ischemic stroke and their predicted gene targets. PLoS One. 2014;9:e99283. [PubMed: 24911610]
7. Vijayan M, Reddy PH: Peripheral biomarkers of stroke: Focus on circulatory microRNAs. Biochim Biophys Acta 2016;1862:1984–93. [PubMed: 27503360]
8. He W, Chen S, Chen X, et al.: Bioinformatic Analysis of Potential microRNAs in Ischemic Stroke. J Stroke Cerebrovasc Dis 2016;25:1753–9. [PubMed: 27151415]
9. Jiugeng Feng RF, Chunhui Zeng, Minjun Wei & Tao Hong: MicroRNA-664 regulates cell invasion and migration and epithelial-mesenchymal transition by targeting TGF-β signal in glioblastoma. Int J Clin Exp Pathol 2016;9:10.
10. Ander BP, Barger N, Stamova B, et al.: Atypical miRNA expression in temporal cortex associated with dysregulation of immune, cell cycle, and other pathways in autism spectrum disorders. Mol Autism 2015; 6:37. [PubMed: 26146533]
11. Kim YW, Kim EY, Jeon D, et al.: Differential microRNA expression signatures and cell type-specific association with Taxol resistance in ovarian cancer cells. Drug Des Devel Ther 2014;8:293–314.

12. Katayama Y, Maeda M, Miyaguchi K, et al.: Identification of pathogenesis-related microRNAs in hepatocellular carcinoma by expression profiling. Oncol Lett 2012; 4:817–823. [PubMed: 23205106]
13. Wang R, Dong LD, Meng XB et al. Unique MicroRNA signatures associated with early coronary atherosclerotic plaques. Biochem Biophys Res Commun 2015; 464:574–9. [PubMed: 26159918]
14. Wang W, Zhang Y, Zhu B, et al. Plasma microRNA expression profiles in Chinese patients with rheumatoid arthritis. Oncotarget 2015;6:42557–68. [PubMed: 26637811]
15. Laudanski P, Charkiewicz R et al. Profiling of Selected MicroRNAs in Proliferative Eutopic Endometrium of Women with Ovarian Endometriosis. Biomed Res Int 2015;760698. [PubMed: 26366419]

Table 1.

Patient demographics.

Category	**Total (n=16)**
Age, mean (range)	74.3 (56, 91)
Sex (% Male)	8 (50%)
Stroke Risk Factors	
Hypertension	93.6 %
Coronary Artery Disease	50.0 %
Diabetes	31.2 %
Smoking	18.7%
Hyperlipidemia	75.0%
Race	
Caucasian	62.5 %
African American	12.5 %
Hispanic	6.25 %
Asian	6.25 %
Other	12.5 %
Stroke location	
Left MCA	50%
Right MCA	50%

Table 2.

Differential miRNA expression between controls (n=8) and all stroke cases (n=16). Known disease associations are listed.

miRNA	P-value	Log FC	Fold change	Regulation	Previous Associations
hsa-miR-145–5p	0.0398	0.399	1.319	Upregulated	Ischemic Stroke [3–5,7,8]
hsa-miR-943	0.0437	0.234	1.176	Upregulated	Cancer [2], Ischemic Stroke [5,7]
hsa-miR-664a-3p	0.0247	0.227	1.170	Upregulated	Cancer [9]
hsa-miR-2116–5p	0.0437	−0.179	0.883	Downregulated	Cancer [12]
hsa-miR-4531	0.0428	−0.194	0.874	Downregulated	None
hsa-miR-4764–5p	0.0419	−0.251	0.840	Downregulated	Rheumatoid Arthritis [14]
hsa-miR-647	0.0247	−0.283	0.822	Downregulated	Cancer [11]
hsa-miR-4742–3p	0.0383	−0.289	0.818	Downregulated	Autism [10]
hsa-let-7e-5p	0.0398	−0.309	0.807	Downregulated	Ischemic Stroke [3–5]
hsa-miR-5584–3p	0.0247	−0.312	0.806	Downregulated	None
hsa-miR-4756–3p	0.023	−0.333	0.794	Downregulated	None
hsa-miR-4709–3p	0.023	−0.372	0.773	Downregulated	Autism [10]
hsa-miR-5187–3p	0.0113	−0.426	0.744	Downregulated	Endometriosis [15]
hsa-miR-1273e	0.0113	−0.426	0.744	Downregulated	Ischemic Stroke [5]

Table 3.

Additional differentially expressed microRNA between severe (NIHSS > 10) stroke (n=10) and control patients (n=8). Known disease associations are listed.

miRNA	P-value	Log FC	Fold change	Regulation	Previous Associations
hsa-miR-29a-5p	0.022	0.279	1.214	Upregulated	Atherosclerosis [13]
hsa- miR-151a-3p	0.023	0.674	1.596	Upregulated	Ischemic Stroke [7]
hsa-miR-487b-3p	0.025	0.524	1.438	Upregulated	Ischemic Stroke [6,7]
let-7b-3p	0.046	0.420	1.338	Upregulated	Ischemic Stroke [5,7]
hsa-miR-4531	0.023	−0.221	0.858	Downregulated	None
hsa-miR-15b-5p	0.048	−0.560	0.678	Downregulated	Atherosclerosis [13], Ischemic Stroke [7]