MicroRNA Profile in Cerebrospinal Fluid and Plasma of Patients with Spontaneous Intracerebral Hemorrhage

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Spontaneous intracerebral hemorrhage (ICH) accounts for 15–20% of “acute stroke” with a significant morbidity and mortality [1]. Following ICH, pathobiological processes (blood–brain barrier (BBB) damage, cerebral edema, and inflammation) are activated [1,2]. The molecular changes associated with these processes could be a target for therapeutic interventions or potential biomarkers.

Gene expression profiling of human tissues and experimental models of ICH have identified potential candidate genes [3]. MicroRNAs (miRNAs) are important gene regulators and primarily identified in tissues encapsulated in microvesicles [2,4]. Following tissue injury, miRNAs are released into the extracellular space [4]. Comparison of miRNAs from cerebrospinal fluid (CSF) and blood from the same patient is unique as CSF likely reflects the local events of injured brain tissue compared to miRNAs in the bloodstream.

This study was approved by the Ochsner Medical Center Institutional Review Board. Patients with ICH confirmed on computed tomography (CT) scan who required a ventricular drain were consented for CSF and blood sample collection. We enrolled five male subjects with a median age of 54 years (range, 37–78 years). The basic clinical information is provided (Table S1). Blood samples (10 mL) were collected in sodium citrate tubes, and CSF samples (3–5 mL) were collected within 24 h of presentation. The study was designed such that each patient serves as an intrinsic control in comparing CSF and blood miRNA expression patterns.

Total RNA containing miRNAs was isolated from plasma and CSF (200 μ L) for profiling of 782 known miRNAs (Exiqon). The real-time PCR was run for 40 cycles in a CFX-384 thermal cycler (Bio-Rad, Hercules, CA, USA). Cycle threshold (Ct) data were obtained using the regression method. Data were normalized

using the global mean and log₂-transformed using the GenEx software (Exiqon, Vedbaek, Denmark). Using a Ct value cutoff of 39, 423 miRNAs were reliably expressed in CSF and plasma. Of these, 128 miRNAs were detected in CSF, 14 miRNAs in plasma, and 281 miRNAs in both CSF and plasma. Differential miRNA expression analysis found 242 significantly expressed miRNAs ($P < 0.05$). Of these, 139 miRNAs were found at 2-fold higher in CSF and 94 miRNAs at 2-fold lower in CSF compared to plasma. When the P -values were adjusted for multiple testing correction using the Benjamin–Hochberg method, 211 miRNAs were statistically significant ($P < 0.05$; Table S2).

The heat map of the top 25 significantly expressed miRNAs is shown with the corresponding fold change and significance value (Figure 1A, B). The highly expressed miR-204-5p (2075-fold), miR-125b-5p (108-fold), miR-9-5p (299-fold), miR-338-3p (146-fold), miR-187-3p (21-fold), and miR-9-3p (42-fold) in CSF are associated with the regulation of matrix metalloproteinase-9 (MMP-9), IL-1 β , IL-6, occludin, and SELE, to name a few. In the case of miR-204-5p, miR-9-3p, and miR-338-3p, which target MMP-9, their expressions in plasma were decreased, suggesting an increased expression and activation of pro-MMP-9 in plasma. However, the expression in CSF is decreased. In animal and human studies, elevated MMP-9 in plasma during the acute phase of ICH was associated with increased BBB damage, hematoma growth, and perihematomal edema [5].

MiR-126-5p expression was downregulated (–8.3-fold) in CSF compared to plasma. miRNA-126 regulates vascular cell adhesion molecule 1 (VCAM-1) expression in endothelial cells. Decreased expression of miRNA-126 may lead to VCAM-1 expression and promotes immune cell localization at the site of injury. Zhu et al. [6] found miRNA levels in serum were mostly downregulated

Figure 1 (A) Heatmap hierarchical cluster of the top 25 significantly expressed miRNAs; and (B) the top 25 significantly expressed miRNAs and the fold change in CSF relative to plasma. P-values reported are with Benjamin-Hochberg multiple test correction.

compared to healthy controls and that the serum level of miR-126 correlated with perihematomal edema. On the contrary, Guo et al., [4] found upregulation of miRNAs associated with inflammation in plasma.

In healthy subjects, the numbers of miRNA in plasma are generally higher and unique compared with CSF [7]. We found large increases in miRNA, both in abundance and fold change, in CSF than plasma. miRNA profile in CSF is physiologically contiguous with profiles in brain extracellular fluid [8]. Hence, the differences in the miRNA content between CSF and plasma may reflect an altered systemic inflammatory response leading to neuroinflammation and BBB damage.

Pathway enrichment analysis of significant miRNAs using Diana-miRPath v2.0 [9] and the Kyoto Encyclopedia of Genes and Genomes (KEGG) identified pathways that may be activated in acute ICH. For the 128 miRNAs detected only in CSF, the top over-represented KEGG pathways implicated in BBB disruption include PI3K-Akt signaling, MAPK signaling, regulation of actin cytoskeleton, focal adhesion, endocytosis, TGF- β signaling, gap, and adherens junctions. Predicted and validated target genes regulated by miRNAs were determined using the DIANA-TarBase v6.0 (Athena, Athens, Greece). Pathway enrichment analysis of the 139 miRNAs in CSF and 94 miRNAs in plasma found a similar pathway profile as those identified for CSF-specific miRNAs (Table 1A). The miRNA-regulated genes associated with these pathways were

identified using the TarBase database of experimentally validated targets. Focal adhesion and neuroactive ligand receptor signaling were represented for upregulated miRNAs in CSF, whereas axon guidance and cytokine receptor signaling were represented by downregulated miRNAs. The validated targets for some of these miRNAs are associated with neuroinflammation (Table 1B). Previous studies have implicated the MAP kinase, Akt, and NF κ B signaling pathways and the involvement of these cytokines in neuroinflammation [5,10]. Although the miRNA profiles in CSF and plasma are distinct, the upregulated miRNAs in CSF and plasma regulate the same group of genes and shared many common signaling pathways associated with neuroinflammation and BBB damage. Therefore, systemic and intracerebral pathological conditions are reflective of one another, and that systemic monitoring may help guide intervention for brain injury after ICH. Hence, our study suggests a possible role for miRNA in the genetic regulation of these pathways and cytokines. Given the small sample size, we are not able to account for potential variations in miRNA profiles due to race, gender, or differences in healthy individuals.

In conclusion, the present study identified a significantly greater number of miRNAs in the CSF than plasma of ICH patients. The large fold decrease in the plasma miRNAs that regulate inflammation-associated genes suggests that the systemic regulation of these genes may be affected. These miRNAs

Table 1 Significantly expressed miRNAs found in CSF and plasma and their associated KEGG pathways. (A) The numbers of miRNAs that were found significantly expressed versus the total number of miRNAs associated with a KEGG pathway; (B) mRNA targets of the significantly expressed miRNAs and fold change relative to plasma

A. Pathways and significantly expressed miRNAs		
Pathway ^a	miRNA in plasma ^b	miRNA in CSF ^b
Adherens junctions	16/142	17/142
MAPK signaling	33/116	31/116
WNT signaling	22/104	26/104
PI3K-Akt signaling	35/197	39/197
Focal adhesion	82/1593	105/1593
Actin cytoskeleton	22/85	23/85
Apoptosis	23/142	24/142
Cytokine receptor signaling	27/98	19/98
Neuroactive ligand receptor signaling	4/35	10/35
Axon guidance	20/664	13/664
Ubiquitin-mediated proteolysis	14/60	14/60
Dopaminergic signaling	16/65	17/65
B. mRNA target: miRNA (Fold change)		
MMP-9: miR-204-5p (2075); miR-338-3p (146); miR-9-5p (299); miR-211-5p (15); miR-29b-3p (6); let-7e-5p(6)		
TNF- α : miR-187-3p (21); miR-143-3p (2); miR-130a-3p (2)		
IL-1 β : miR-204-5p (2075)		
IL-6: miR-125b-5p (108)		
Occludin: miR-9-3p (42); miR-488-3p (12)		
SELE: miR-204-5p (2075); miR-211-5p (15)		
CCR5: miR-125b-5p (108)		
ICAM-1: miR-187-3p (21); miR-155-5p (-13); miR-31-5p (3); miR-221-3p (-3); miR-222-3p (-3)		
CXCL8: miR-302a-3p (21)		
CXCL5: miR-488-3p (13); miR-577 (16)		
CXCL10: let-7a-2-3p (12)		

^aPathways that were found significant based on a threshold cutoff ($P < 0.05$) with FDR correction using Diana-TarBase v6.0 and miRPath default setting. ^bThe number of input miRNA to the number of miRNA represented in the respective KEGG pathway Akt, Ak strain transforming; KEGG, Kyoto Encyclopedia of Gene and Genome; MAPK, mitogen-activated protein kinase; miRNA, microRNA; PI3K, phosphoinositide-3-kinase; Wnt, wingless-type MMTV integration site family, CXCL5, chemokine (C-X-C motif) ligand 5; CXCL8, chemokine (C-X-C motif) ligand 8 (Interleukin 8); CXCL10, chemokine (C-X-C motif) ligand 10; ICAM-1, intercellular adhesion molecule-1; IL-1 β , interleukin-beta-1; IL-6, interleukin-beta-6; MMP-9, metalloproteinase-9; SELE, selectin E.

can provide insights on the underlying neuroinflammation in ICH.

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Conflict of interests

The authors declare that they have no competing interests. No portion of this study has been published or is under consideration for publication elsewhere. The authors have no personal, financial, or institutional interests in any of the drugs and materials described in this manuscript.

References

- Keep RF, Hua Y, Xi G. Intracerebral haemorrhage: mechanisms of injury and therapeutic targets. *Lancet Neurol* 2012;**11**:720–731.
- Zheng HW, Wang YL, Lin JX, Li N, Zhao XQ, Liu GF. Circulating MicroRNAs as potential risk biomarkers for hematoma enlargement after intracerebral hemorrhage. *CNS Neurosci Ther* 2012;**18**:1003–1011.
- Yang T, Gu J, Kong B, et al. Gene expression profiles of patients with cerebral hematoma following spontaneous intracerebral hemorrhage. *Mol Med Rep* 2014;**10**:1671–1678.
- Guo D, Liu J, Wang W, et al. Alteration in abundance and compartmentalization of inflammation-related miRNAs in plasma after intracerebral hemorrhage. *Stroke* 2013;**44**:1739–1742.
- Florczak-Rzepka M, Grond-Ginsbach C, Montaner J, Steiner T. Matrix metalloproteinases in human spontaneous intracerebral hemorrhage: An update. *Cerebrovasc Dis* 2012;**34**:249–262.
- Zhu Y, Wang JL, He ZY, Jin F, Tang L. Association of altered serum microRNAs with perihematomal edema after acute intracerebral hemorrhage. *PLoS One* 2015;**10**:e0133783.
- Webber JA, Baxter DH. The microRNA spectrum in 12 bodily fluids. *Clin Chem* 2010;**56**:11 (1733–1741).
- Alexandrov PN, Dua P, Hill JM, Bhattacharjee S, Zhao Y, Lukiw WJ. microRNA (miRNA) speciation in Alzheimer's

disease (AD) cerebrospinal fluid (CSF) and extracellular fluid (ECF). *Int J Biochem Mol Biol* 2012;**3**:365–373.

9. Vlachos IS, Kostoulas N, Vergoulis T, et al. DIANA miRPath v.2.0: Investigating the combinatorial effect of

microRNAs in pathways. *Nucleic Acids Res* 2012;**40**(Web Server issue):W498–W504.

10. Ohnishi M, Monda A, Takemoto R, et al. Sesamin suppresses activation of microglia and p44/42 MAPK

pathway, which confers neuroprotection in rat intracerebral hemorrhage. *Neuroscience* 2013;**232**:45–52.

Supporting Information

The following supplementary material is available for this article:

Table S1. Patient basic clinical information.

Table S2. Significantly expressed miRNA in CSF vs plasma.