UC Davis
UC Davis Previously Published Works

Title

MicroRNA and their target mRNAs change expression in whole blood of patients after intracerebral hemorrhage.

Permalink

https://escholarship.org/uc/item/9pp7c9f7

Journal

Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism, 40(4)

ISSN

0271-678X

Authors

Cheng, Xiyuan
Ander, Bradley P
Jickling, Glen C
et al.

Publication Date

2020-04-01

DOI

10.1177/0271678x19839501

Peer reviewed

MicroRNA and their target mRNAs change expression in whole blood of patients after intracerebral hemorrhage

**Xiyuan Cheng[1,2], Bradley P Ander[1], Glen C Jickling[1],
Xinhua Zhan[1], Heather Hull[1], Frank R Sharp[1,2,*]
and Boryana Stamova[1,*]**

Abstract

Previous studies showed changes in mRNA levels in whole blood of rats and humans, and in miRNA in whole blood of rats following intracerebral hemorrhage (ICH). Thus, this study assessed miRNA and their putative mRNA targets in whole blood of humans following ICH. Whole transcriptome profiling identified altered miRNA and mRNA levels in ICH patients compared to matched controls. Target mRNAs of the differentially expressed miRNAs were identified, and functional analysis of the miRNA-mRNA targets was performed. Twenty-nine miRNAs (22 down, 7 up) and 250 target mRNAs (136 up, 114 down), and 7 small nucleolar RNA changed expression after ICH compared to controls (FDR < 0.05, and fold change ≥ |1.2|). These included Let7i, miR-146a-5p, miR210-5p, miR-93-5p, miR-221, miR-874, miR-17-3p, miR-378a-5p, miR-532-5p, mir-4707, miR-4450, mir-1183, Let-7d-3p, miR-3937, miR-4288, miR-4741, miR-92a-1-3p, miR-4514, mir-4658, mir-3689d-1, miR-4760-3p, and mir-3183. Pathway analysis showed regulated miRNAs/mRNAs were associated with toll-like receptor, natural killer cell, focal adhesion, TGF-β, phagosome, JAK-STAT, cytokine–cytokine receptor, chemokine, apoptosis, vascular smooth muscle, and RNA degradation signaling. Many of these pathways have been implicated in ICH. The differentially expressed miRNA and their putative mRNA targets and associated pathways may provide diagnostic biomarkers as well as point to therapeutic targets for ICH treatments in humans.

Introduction

Intracerebral hemorrhage (ICH) has a high morbidity and mortality rate.[1,2] ICH evokes local and systemic inflammation, in which blood cells form leukocyte-platelet aggregates, adhere to brain endothelial cells, and infiltrate brain, resulting in blood–brain barrier (BBB) disruption, brain edema and parenchymal brain injury that worsen outcomes.[3]

To assess the peripheral inflammatory response to ICH, we first showed that the expression of a group of mRNA transcripts in whole blood of rats with experimental ICH was different from controls and ischemic stroke.[4] To address one mechanism for these mRNA changes, we studied microRNA (miRNA) because they are short non-coding RNA that bind to complementary sequences in the 3′ untranslated region

(3′UTR) of mRNA of hundreds of target genes, resulting in transcript degradation and post-translational suppression of target genes.[5–7] We found miRNA expression changed in whole blood of rats following ICH, and the changes differed from ischemic stroke and controls.[8] These data provide a new way to

[1]Department of Neurology, University of California at Davis, Sacramento, CA, USA
[2]Toxicology and Pharmacology Graduate Program, University of California at Davis, Davis, CA, USA

*These authors contributed equally to this work.

Corresponding author:
Xiyuan Cheng, Department of Neurology, University of California, 2805 50th Street, Sacramento, CA 95817, USA.
Email: yuacheng@ucdavis.edu

understand the immunological mechanisms relevant to ICH and stroke on a genome-wide scale.[9,10]

To extend these animal studies, we have recently described changes of mRNA expression in whole blood of humans related to inflammation, immune responses, clotting and other pathways after ICH.[11,12] As in the case of the animal studies, the mechanisms for these mRNA changes were not known. Thus, in this study we examine miRNA in whole blood of ICH patients and identify their potential mRNA target genes. Although there are less than 3000 annotated miRNAs in the human genome,[13] it is estimated that they modulate over 60% of the ~20,000 human protein-coding genes.[14,15]

It is important to note that miRNA have been examined in serum and plasma in many studies of ICH and subarachnoid hemorrhage.[16–25] However, the miRNA identified in those serum/plasma studies are extracellular, and likely associated with exosomes and/or bound to Argonaute[26] and have uncertain mRNA/gene targets. For the current study, we chose to study whole blood, where the miRNA and mRNA would be primarily intracellular within leukocytes, red cells, platelets and other blood cells. Thus, these data add to a growing literature on the expression of miRNA and their putative mRNA targets change in human whole blood following ICH. They could provide diagnostic biomarkers as well as therapeutic targets for future study.

Materials and methods

Study subjects

Patients were recruited between 2005 and 2013 from the Universities of California at Davis and San Francisco. The protocol was approved by the UC Davis and UC San Francisco Institutional Review Boards and adheres to all federal and state regulations related to the protection of human research subjects, including The Common Rule, the principles of The Belmont Report, and Institutional policies and procedures. Written informed consent was obtained from all participants or their proxy. There were 23 ICH patients, and 17 vascular risk factor (VRF) controls (Table 1). ICH diagnoses were made by two board-certified neurologists based upon medical history, exams, and computed tomographic (CT) brain scan and/or magnetic resonance imaging (MRI). The enrollment criteria for vascular risk factor controls (VRFCs) were subjects diagnosed with hypertension, hyperlipidemia, atrial fibrillation or diabetes, but not having a prior stroke, myocardial infarction, or transient ischemic attack (TIA). VRFC were also matched to ICH patients for age, sex, race, diabetes, hypertension, hyperlipidemia, and atrial fibrillation. No ICH patient had a cerebral cavernoma angioma (CCM), arterial venous malformation (AVM), abnormal platelet count or anticoagulants as the cause of their ICH.

One blood sample was obtained from each ICH subject after brain hemorrhage onset which varied from 4.2 to 101.3 h. PAXgene tubes (Qiagen) were used to collect and lyse all blood cells and to stabilize RNA immediately in blood. The tubes were left at room temperature for 2 h, and then stored frozen at $-80°$C until processed.

Total RNA isolation

Total RNA was isolated according to the manufacturer's protocol (PAXgene blood miRNA kit; PreAnalytiX). RNA quantity and quality were determined using a Nano-Drop (Thermo Fisher) and Agilent 2100 Bioanalyzer, respectively. Samples required A_{260}/A_{280} absorbance ratios ≥ 1.8, a 28S/18S rRNA ratio ≥ 1.8,

Table 1. Demographic variables for ICH patients compared to VRF controls.

Variables	VRF control (n = 17)	ICH ≤ 72 h (n = 14)	P-value (ICH ≤ 72 h vs. VFR controls)	ICH > 72 h (n = 9)	P-value (ICH > 72 h vs. VFR controls)
Age (years), mean (SD)	62.8 (10.7)	67.9 (14.9)	0.27	59.9 (14.2)	0.57
Race Caucasian, n (%)	10 (59%)	7 (50%)	0.72	2 (22.2%)	0.11
Gender Male, n (%)	9 (67%)	11 (79%)	0.26	7 (77.8%)	0.4
Hypertension, n (%)	14	13	0.61	6	0.63
Diabetes, n (%)	4	3	1	2	1
Hyperlipidemia, n (%)	9	3	0.14	2	0.22
Atrial fibrillation, n (%)	3	1	0.61	0	0.53
Hours since ICH, mean (SD)	N/A	40.8 (17.6)	0.0001	86.6 (9.90)	0.0001

Note: P-values represent the comparisons of either ICH ≤ 72 h vs. VRFC or ICH > 72 h vs. VRFC using t-test, Chi-square or Fisher's exact test where appropriate. ICH: intracerebral hemorrhage; VRF: vascular risk factor.

and an RNA integrity number (RIN) ≥ 8. Of the total RNA isolated, half was used for miRNA arrays, and the other half was used for mRNA arrays. This ensures the miRNA and their target mRNAs are being assessed from the same batch of total RNA for each subject.

mRNA microarrays

Total RNA (80 ng each sample) was converted to cDNA and amplified using the ApplauseWT-Amp Plus ST System (NuGEN, San Carlos, CA) and labeled using the FL-Ovation cDNA Biotin Module V2 (NuGEN). The product was then hybridized to Affymetrix HTA 2.0 Gene Chip microarrays (Affymetrix, Santa Clara, CA) to profile gene expression of mRNAs according to the manufacturer protocol. Though exons are included on the HTA 2.0 arrays, the data here were analyzed at the "whole gene" level, which entailed using the average expression of the exon and junction probe-sets for each gene.

miRNA arrays

Isolated total RNA was processed on Affymetrix Gene Chip miRNA 3.0 Arrays (Affymetrix, Santa Clara, CA). For each subject, 200 ng of total RNA was used per microarray. RNA was labeled using FlashTag Biotin HSR labeling kits (Affymetrix, CA) with no amplification, hybridized to microarrays, and scanned using an Affymetrix GCS3000 Gene Array Scanner according to the manufacturer's protocol.

Statistical analysis of mRNA and miRNA data

Raw gene expression data were normalized using robust multichip averaging (RMA) using Partek Flow and Partek Genomics Suite version 7 (Partek Inc., St. Louis, MO). Statistical analyses were performed on mRNA or miRNA data with two comparisons dependent on time of blood draw following stroke: (1) ICH ≤ 72 h ($n = 14$) vs. VRFC ($n = 17$); (2) ICH > 72 h ($n = 9$) vs. VRFC ($n = 17$). We used 72 h as a cutoff in order to characterize acute (≤ 72 h) and subacute/chronic changes (> 72 h). Univariate analysis (t-test, Chi-square or Fisher's exact test) was performed to identify potential confounding factors, including gender, age, race, and VRFs that differ between ICH groups and VRFC to avoid RNA expression differences due to unmatched samples. Since no factor had a $P < 0.05$, none of these were included in the ANOVA model. An ANOVA model that included diagnosis and technical variation (array batch) was employed with a False discovery rate (FDR) to correct for multiple-comparisons. miRNA or mRNA with a FDR < 0.05 and fold change $\geq |1.2|$ were considered significant.

Integrated mRNA–miRNA functional analyses and cross-validation analysis

The integrated mRNA–miRNA functional network analyses were performed as previously described with minor modifications.[27] Briefly, the lists of regulated miRNAs and regulated mRNAs in blood of ICH patients were loaded into the functional network analyses software: Exploratory Gene Association Networks (EGAN).[28] After identifying miRNA-mRNA pairs using EGAN built-in miRNA-targets database, including TargetScan Human 7.0,[29] and miRTarBase,[30,31] we performed functional analysis of the paired miRNAs and mRNAs using Kyoto Encyclopedia of Genes and Genomes (KEGG). Cross-validation of identified miRNAs or mRNAs was performed to determine prediction accuracy of the optimal model using forward selection k-nearest neighbor algorithm (k=1,3,5,7,9,11,13) in Partek Genomics Suite version 7.[32] Sensitivity and specificity of the best classifier were calculated.

Results

Demographic and clinical characteristics

Of the 40 subjects in the study, the average age (years, $\pm$ SD) was 63.9 ± 13.1, and 27 (67.5%) were male. The study was racially/ethnically mixed with 19 (47.5%) Caucasian, 8 (20%) Hispanic, 2 (5%) African American, 5 (12.5%) Asian, and 6 (15%) other races. Demographic and clinical characteristics are listed in Table 1 and are broken down into the two comparison subgroups: ICH ≤ 72 h vs. VRFC and ICH > 72 h vs. VRFC. There were no significant differences in subjects' demographics including age, gender, race, hypertension, hyperlipidemia, diabetes, and atrial fibrillation between the two ICH groups and VRFC (Table 1).

Of the total ICH patients ($n = 23$), hypertension was the cause of ICH in 78.3% of patients (deep location with hypertension), 17.45% were due to amyloid angiopathy (lobar/cortical with microbleeds) and 4.35% were cryptogenic. There were no significant differences in causes of ICH (P-value = 0.34) between ICH ≤ 72 h and ICH > 72 h in terms of hypertension and non-hypertension. In the ICH ≤ 72 h group ($n = 14$), 71.4% of patients had hemorrhage in deep area and 28.6% in lobar area. In ICH > 72 h group ($n = 9$), 55.6% of patients had hemorrhage in deep area and 44.4% in lobar area. There were no significant differences in ICH location (P-value = 0.66) between ICH ≤ 72 h and ICH > 72 h in terms of deep and lobar area; 21 out of 23 ICH patients received anti-hypertensive drugs including the β-blocker labetalol and/or several different calcium-channel blocker drugs within 72 h of hospital admission.

Table 2. The altered miRNAs in blood at $\leq$ 72 h of ICH patients compared to vascular risk factor controls.

miRNA	FDR *P*-value (ICH $\leq$ 72 h vs. CTRL)	Fold change (ICH $\leq$ 72 h vs. CTRL)
miR-210-5p	3.20E-02	−2.29
miR-17-3p	3.43E-02	−2.16
let-7i	2.84E-02	−1.9
miR-378d	2.51E-02	−1.85
miR-378i	2.42E-02	−1.78
miR-874	3.86E-02	−1.77
miR-378c	2.28E-02	−1.75
miR-221	2.08E-03	−1.73
miR-378a-5p	2.28E-02	−1.67
miR-146a-5p	3.87E-02	−1.61
miR-378e	3.40E-02	−1.56
miR-532-5p	4.77E-02	−1.52
miR-93-5p	4.59E-02	−1.38
mir-4707	1.50E-02	−1.27
miR-4450	4.22E-02	−1.23
mir-1183	4.77E-02	1.22
let-7d-3p	3.32E-02	1.92

Note: *P*-values represent the comparisons of ICH $\leq$ 72 h vs. VRFC using ANOVA with FDR <0.05 and fold change > |1.2|. FDR: False discovery rate correction for multiple comparisons; ICH: intracerebral hemorrhage; CTRL: control.

The hypertensive controls were on similar classes of anti-hypertensive drugs.

Differentially expressed miRNAs and mRNAs

We measured miRNA expression in blood samples of three groups (ICH at $\leq$ 72 h, $n = 14$; ICH at > 72 h, $n = 9$; and VRFC, $n = 17$) using Affymetrix Gene Chip miRNA 3.0 Arrays. There were 29 miRNAs (22 down, 7 up) that were significantly regulated either at $\leq$ 72 h (Table 2) or > 72 h (Table 3) after ICH compared to VRFC (FDR < 0.05, fold change $\geq$ |1.2|).

The 15 down-regulated miRNAs at $\leq$ 72 h included: miR-146a-5p, miR-210-5p, miR-93-5p, miR-17-3p, miR-378a-5p, miR-221, let-7i, miR-378c, miR-378d, miR-378e, miR-378i, miR-532-5p, miR-874, miR-4450, and mir-4707 (Table 2, Figure 1(a)). The two up-regulated miRNAs at $\leq$ 72 h were mir-1183 and let-7d-3p (Table 2, Figure 1(a)). In addition, three small nucleolar RNAs (snoRNA) were differentially expressed at $\leq$ 72 h, including SNORA31B (*P*-value = 5.68E-05, fold change=1.25), SNORD45 (*P*-value = 1.36E-04, fold change = 1.29), and SNORA15 (*P*-value = 3.54E-04, fold change = 1.38).

The seven down-regulated miRNA at > 72 h included: miR-92a-1-3p, miR-221, miR-3937, miR-4288, miR-4514,

Table 3. The altered miRNAs in blood at >72 h of ICH patients compared to vascular risk factor controls.

miRNA	FDR *P*-value (ICH > 72 h vs. CTRL)	Fold change (ICH > 72 h vs. CTRL)
miR-3937	3.25E-02	−2.75
miR-221	5.55E-05	−2.13
miR-4288	3.17E-02	−1.87
mir-4741	4.93E-02	−1.48
miR-92a-1-3p	4.19E-02	−1.4
mir-4707	4.86E-02	−1.26
miR-4514	4.93E-02	−1.24
mir-4658	3.17E-02	1.23
mir-3689d-1	4.86E-02	1.24
miR-4760-3p	4.93E-02	1.25
mir-3183	4.19E-02	1.26
let-7d-3p	3.42E-02	2.22

Note: *P*-values represent the comparisons of ICH >72 h vs. VRFC using ANOVA with FDR <0.05 and fold change > |1.2|. FDR: false discovery rate correction for multiple comparisons; ICH: intracerebral hemorrhage; CTRL: control.

mir-4707 and mir-4741 (Table 3, Figure 1(b)). The five up-regulated miRNA at > 72 h were mir-4658, mir-3689d-1, miR-4760-3p, mir-3183, and let-7d-3p (Table3, Figure 1(b)). Four snoRNA that were differentially expressed at >72 h included: SNORD115-13 (*P*-value = 1.75E-04, fold change = 1.20), and three snoU13 paralogs on chromosome 12 (*P*-value = 5.42E-05, fold change = 1.21), chromosome 9 (*P*-value = 1.82E-04, fold change = 1.22), and chromosome 15 (*P*-value = 6.12E-05, fold change = 1.23). One scaRNA was elevated at >72 h, mgU2-19/30 (*P*-value = 1.76E-04, fold change = 1.21).

We also measured mRNA expression using the same blood samples on Affymetrix HTA 2.0 Gene Chip microarrays. There were 250 mRNAs (136 up and 114 down) that significantly changed expression at $\leq$72 h following ICH compared to VRFC (FDR < 0.05, fold change $\geq$ |1.2|) (Figure 2, Supplementary Table 1). No mRNAs were significantly regulated at >72 h after ICH compared to VRFC (FDR < 0.05, fold change $\geq$ |1.2|), likely because of stringent statistics and small numbers of subjects (see below).

Functional analysis of down-regulated mRNAs and up-regulated miRNAs for ICH at $\leq$ 72 h compared to VRFC

Five down-regulated miRNAs (miR-146a-5p, miR-210-5p, miR-93-5p, miR-17-3p, and miR-378a-5p) were paired with up-regulated mRNA that are putative targets of these down-regulated miRNAs in whole blood at $\leq$72 h after ICH using EGAN built-in
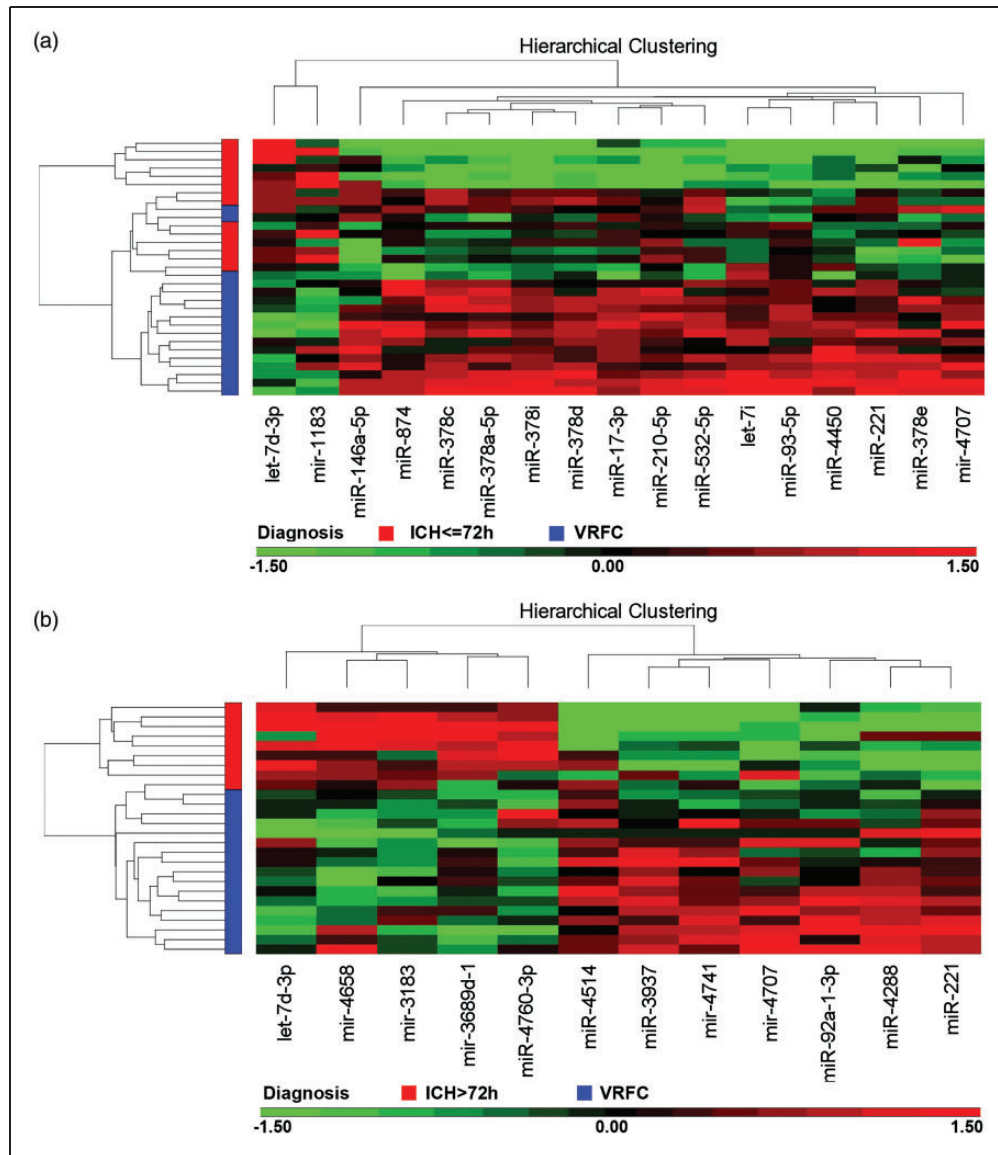
Figure 1. (a) A total of 17 (2 up-regulated, 15 down-regulated) microRNAs (miRNAs) are significantly altered at ≤72 h in whole blood of intracerebral hemorrhage (ICH) patients versus vascular risk factor control patients (VRFC) (FDR < 0.05, fold change ≥ |1.2|). Patients are on the Y axis (Red = ICH ≤ 72 h; Blue = VRFC), and the miRNA are on the X axis (Red = up-regulation; black = no change; green = down-regulation). (b) A total of 12 (5 up-regulated, 7 down-regulated) miRNAs are significantly altered at >72 h in whole blood of ICH patients versus VRFC (FDR < 0.05, fold change ≥ |1.2|). Patients are on the Y axis (Red = ICH > 72 h; Blue = VRFC), and the miRNA are on the X axis (Red = up-regulation; black = no change; green = down-regulation).

miRNA-targets database. The integrated mRNA–miRNA functional analyses showed the signaling networks between the five down-regulated miRNAs and up-regulated mRNAs (BCL2A1, TNFRSF10A, ROCK1, TLR2, TLR6, SPTLC2, ZNF143, CAPZA2, SH3GLB1, ACSL4, SERINC1, KIAA0232, SPOPL, STK17B, RAB8B, ZNF33A, ZBTB34, DCP2, TLR1, TLR5, JAK2, IFNGR1, and others) (Figure 3). The top functional pathways included: toll-like receptor, natural killer cell, focal adhesion, TGF-beta, adipocytokine, fatty acid metabolism, cytokine–cytokine receptor, chemokine, apoptosis, vascular smooth muscle, phagosome, JAK-STAT, and RNA degradation signaling (Figure 3, Supplementary Table 2). An unexpected and unexplained finding was the many pathways for infectious diseases including bacteria, viruses and parasites (Supplementary Table 2).

Cross-validation used all 17 miRNAs differentially expressed at ICH ≤72 h to build a predictive model. The best model was able to discriminate ICH at ≤72 h from VRFC with a normalized correct rate of 83.4% using forward selection with the k-nearest
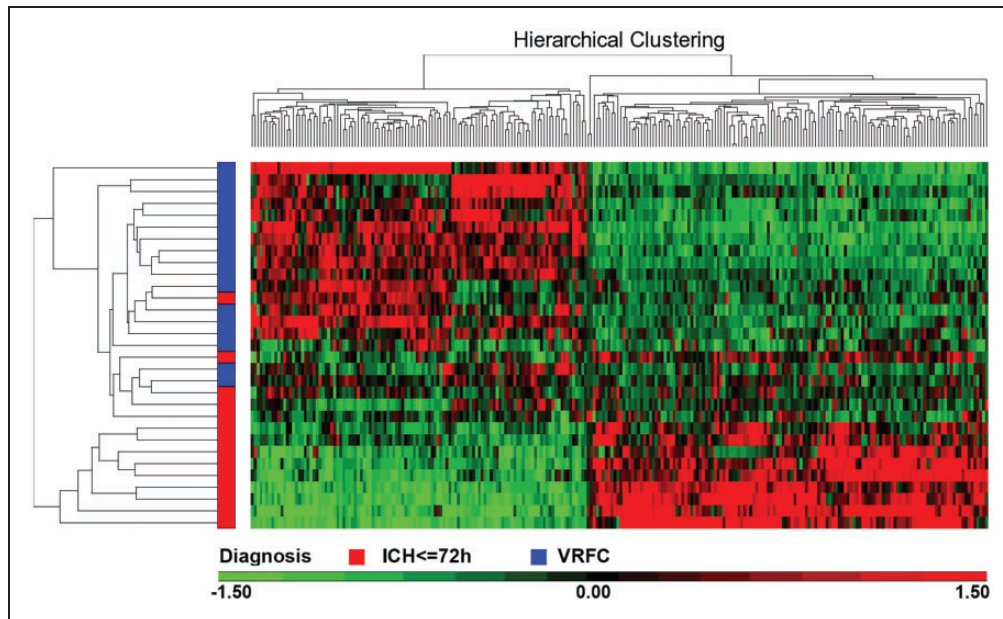
Figure 2. A total of 250 (136 up-regulated, 114 down-regulated) mRNAs changed expression at ≤ 72 h (FDR<0.05; fold change $\geq |1.2|$). Patients are on the Y axis (Red = ICH ≤ 72 h; Blue = VRFC), and the miRNA are on the X axis (Red = up-regulation; black = no change; green = down-regulation).
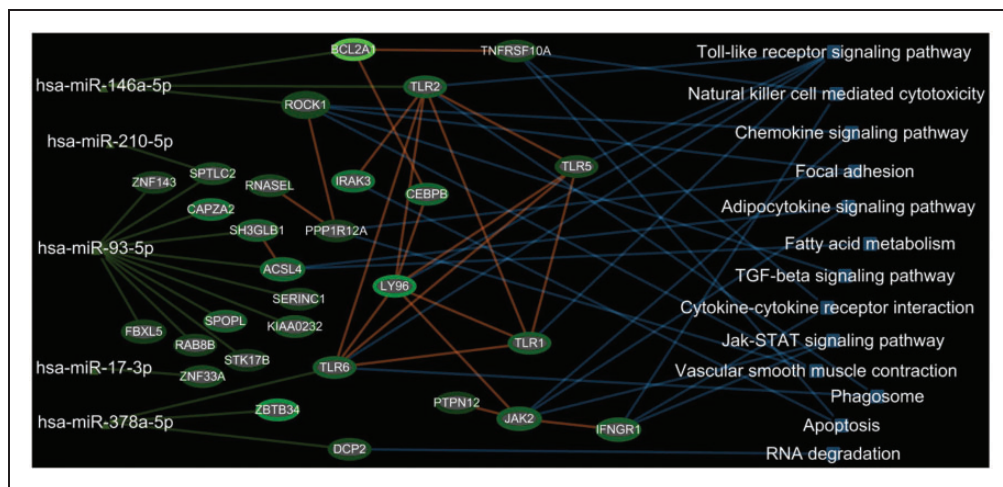

Figure 3. Top functional pathways for the 15 down-regulated miRNAs and the 136 up-regulated mRNAs in blood at ≤ 72 h in ICH patients. Green edges represent interaction of miRNA and mRNA/proteins. Red edges represent known protein–protein interactions. Blue edges connect the mRNA/proteins to enriched KEGG pathways.

neighbor algorithm (k=3). The sensitivity was 78.6% and specificity was 88.2% for ICH at ≤ 72 h. Eight miRNAs were identified for the best predictive model, including mir-1183, mir-4707, let-7d-3p, let-7i, miR146a-5p, miR-17-3p, miR-210-5p and miR-221. The mRNA predictive model that was able to best discriminate ICH at ≤ 72 h from VRFC was generated using a k-nearest neighbor algorithm (k=13) with a normalized correct rate of 83.4% using 43 genes.

The sensitivity was 88.2% and specificity was 88.2% for ICH at ≤ 72 h. Future studies will need to perform predictions on independent cohorts as a function of time after ICH.

Discussion

There are several important findings from this study. First, of the 17 miRNA regulated at ≤ 72h following

ICH, 15 were down-regulated and 2 were up-regulated. However, of the 12 different miRNA regulated at >72 h following ICH, 7 were down-regulated and 5 were up-regulated. This shows a change of the differentially expressed miRNA in two different groups (at ≤ 72 h and at > 72 h), which means their target genes may change expression accordingly. In addition, there is a shift in down-regulated miRNA (in the ≤ 72 h group) to more up-regulated miRNA (in the > 72 h group). This is important because miRNA usually reciprocally regulate their target mRNA. That is, if a miRNA is down-regulated, this generally results in increased transcription/translation of its target genes, and the opposite if the miRNA is up-regulated.[33,34] A second significant finding in this study was that the miRNA–mRNA targets were regulated in a large number of inflammatory/immune/cell death pathways including TLR, NK cell, focal adhesion, TGF-beta, adipocytokine, phagosome, JAK-STAT, cytokine–cytokine receptor, chemokine and apoptosis signaling which supports a number of our previous ICH findings (discussed below).[11,12]

To the best of our knowledge, this is the first study of miRNA in whole blood following ICH in humans. There are several advantages of using whole blood compared to serum/plasma: (1) whole blood can be processed using the PAXgene blood collection technology that stabilizes all RNA immediately against degradation and environmental disturbance effect[35]; (2) whole blood miRNA would be mostly intracellular and derived from white blood cells, red blood cells, platelets and other cells in whole blood, and those miRNA are relevant to ICH pathophysiology; (3) the concentration of miRNA in whole blood is high making them easier to detect. Though there have been several miRNAs described in serum or plasma following ICH, their amounts could be affected by blood processing, by cell lysis, and variability due to their low levels.[36–38] These serum and plasma miRNAs would be extracellular and associated with exosomes, Argonaute or other molecules that protect against RNAases[16–25] and thus could be important in long-distance communication. There are only a few common miRNA between our whole blood ICH study and the serum/plasma studies, however. miR-146a decreased in serum/plasma following ICH[16,18–20] and in whole blood in this study. miR-92a-1-5p was elevated in CSF and plasma following ICH in one study,[21] but miR-92a-1-3p decreased at > 72 h in whole blood in our current study. There was no overlap of the miRNA regulated in whole blood in this ICH study compared to miRNA regulated in serum/plasma in several other studies following ICH.[17,22,23] The lack of overlap of the intracellular miRNA regulated in this whole blood study and the extracellular miRNA regulated in serum/plasma following ICH may be due at least in part to the extracellular miRNA being derived from other organs including liver, kidney, heart and others.

Few of the miRNA identified in human whole blood here were detected in our previous rat ICH study.[8] miR-146a increased in whole blood in the rat ICH blood clot model, whereas it decreased in whole blood in this human ICH study. miR-221 decreased in the rat ICH and rat thrombin model, and miR-92a decreased in the rat ICH thrombin model; and both miR-221 and miR-92a-1-3p decreased in whole blood in this human ICH study. Thus, at least as far as the peripheral blood miRNA response in rodent ICH models, it produces relatively few of the miRNA changes seen in human ICH. The reasons for this are uncertain but could suggest that intracerebral blood injections in rodents do not adequately model the miRNA response seen in blood in human ICH.

It is notable that let-7i was decreased at less than 72 h following ICH in this study, and let-7i is similarly decreased in blood of humans with ischemic stroke where it is postulated to play an important role in modulating the leukocyte response to ischemic stroke.[39] Thus, let-7i could play a similar role in ICH. The let-7d miRNA was up-regulated in whole blood by ICH both before and after 72 h in this study. In contrast, let-7d miRNA did not change in plasma following ICH or ischemic stroke.[40]

In previous ICH studies, we studied mRNA that showed differential expression of alternatively spliced transcripts in ICH versus VRFC controls.[11,12] In contrast, the present study examined gene-level expression of mRNA which represents the average expression of all of the alternatively spliced transcripts for each gene. Thus, the current study used a different measure of "mRNA expression" than the previous study, though it is plausible to expect some overlap. Indeed, of the 250 mRNA identified as regulated in this ICH miRNA study, and of the 394 alternatively spliced mRNA identified as being differentially expressed in our previous ICH study,[11] a total of 47 overlapped by gene symbol, which is highly significant using a hypergeometric probability test ($P < 1 \times 10^{-16}$). Upregulated mRNA, which would be associated with decreases of miRNA, common between the two studies included: KLHL2, FBXL5, RNU6-226P, RBMS1, SOS2, LMNB1, HK3, GAS7, FAM126B, NFIL3, IRAK3, JAK2, STX3, DCP2, ZNF267, COL4A3BP, CAPZA2, SLC38A2, SLC8A1, S100A9, IFNGR1, ACSL4, NQO2, MCTP1, TLR2, UBE2J1, PGM2, OSBPL8, RP2, AGTPBP1, FAR1P1, EXOC6, MEF2A and HIPK3. Down-regulated mRNA, which would be associated with up-regulated miRNA, in both studies included: ANKH, GRAP2, NOMO2, FUBP3, FAM102A, NELL2, DDI2, LARP1, SPTAN1, WNK1, RP11-423H2.1, PDK2 and TRAJ7.

Using the EGAN built-in miRNA-targets database, we paired each of the down-regulated miRNAs to its putative target genes that were up-regulated in blood of ICH patients. The pairs of down-regulated miRNA and up-regulated mRNA in blood of ICH patients included: miR-146a-5p–TLR2 (Toll-like receptor 2, fold change = 2.17), miR-146a-5p–ROCK1 (rho-associated protein kinase 1, fold change = 1.62), miR-146a-5p–BCL2A1 (Bcl-2-related protein A1, fold change = 5.61), miR-210-5p–SPTLC2 (Serine Palmitoyl transferase Long Chain Base Subunit 2, fold change = 1.62), miR-93-5p–ACSL4 (fatty acid-CoA ligase 4, fold change = 2.42), miR-93-5p–CAPZA2 (Capping Actin Protein Of Muscle Z-Line Subunit Alpha 2, fold change = 2.62), miR-93-5p–STK17B (Serine/Threonine Kinase 17b, fold change = 1.33), miR-93-5p–SPOPL (Speckle Type BTB/POZ Protein Like, fold change = 2.04), miR-93-5p–SH3GLB1 (SH3 Domain Containing GRB2 Like, Endophilin B1, fold change = 2.07), miR-17-3p–ZNF33A (Zinc Finger Protein 33A, fold change = 1.62), miR-378a-5p–TLR6 (Toll-like receptor 6, fold change = 2.05), miR-378a-5p–DCP2 (Decapping mRNA 2, fold change = 1.75), miR-378a-5p–ZBTB34 (Zinc Finger And BTB Domain Containing 34, fold change = 3.22) and others.

Based on the mRNA fold change varying from 1.33 to 5.61, we defined three ranges of target gene fold change (low range:1.2–2, middle range:2–3, top range: ≥ 3). Among the changed target genes, 11 genes (ROCK1, STK17B, RAB8B, FBXL5, and others) fell into the low range, 6 genes (TLR2, ACSL4, CAPZA2, SPOPL, SH3GLB1, TLR6) fell into the middle range and 2 genes (BCL2A1, ZBTB34) fell into the top range. Some genes (BCL2A1, TLR2 and TLR6) up-regulated at higher level were consistent with our previous ICH blood genomic study.[11]

The TargetScan miRNA-target prediction algorithm incorporates current biological knowledge of miRNA-target rules, such as including a seed-match model, evolutionary conservation, and free binding energy.[29,34,41] The binding affinity of a predicted miRNA-target pair is scored using cumulative weighted context score (CWCS), a regression model that is trained on sequence and contextual features of the predicted miRNA::mRNA duplex.[29] Lower CWCS score (negative number) indicates higher binding affinity of a miRNA to its target gene. For example, CWCS of miR-93-5p-FBXL5, miR-93-5p-STK17B, miR-93-5p-SPOPL, and miR-93-5p-RAB8B are −0.46, −0.41, −0.32 and −0.18, respectively (TargetScan 7.0). These data suggest the binding affinity of miR-93-5p to its target genes is decreased orderly (miR-93-5p-FBXL5 > miR-93-5p-STK17B > miR-93-5p-SPOPL > miR-93-5p-RAB8B). Our data showed that fold change of target genes may not be consistent with the affinity

between miRNA and its target gene, because the affinity is mainly determined by complementary base pairing, 3′-UTR length and other factors.[29] Generally, the cut-off value of CWCS is −0.3, with a CWCS value lower than −0.3 indicating high binding affinity of the miRNA-target pair.[34,42,43] However, CWCS score (even lower than −0.3) is insufficient to ensure a miRNA does bind to its predicted target gene, as all algorithm-predicted miRNA-target pairs are putative.

Previous studies demonstrated the binding of miR-146a-5p to 3′UTR of ROCK1 and the binding of miR-146a-5p to 3′UTR of TLR2, using 3′UTR clone-based luciferase reporter assay,[44,45] and have been collected in the miRTarBase that contains 360,000 experimentally validated miRNA-target interactions.[30,31] These data strongly support our findings of miR-146a-5p-ROCK1 and miR-146a-5p-TLR2 interactions in blood of ICH patients, as the reporter assay provides direct evidence of the binding between a miRNA to 3′UTR of its target gene.

Note that there were three miRNAs consistently regulated in ICH at ≤72 h and ICH at >72 h (down-regulated: miR-221 and mir-4707, and up-regulated let-7d-3p). According to TargetScan 7.0, miR-221 and let-7d-3p have common predicted target genes with high binding affinity (CWSC score < −0.3), such as ABCA8 (ATP Binding Cassette Subfamily A Member 8) and BEND6 (BEN Domain Containing 6). However, neither ABCA8 nor BEND6 mRNA was significantly altered in blood of ICH patients. This is possibly because the effect of down-regulated miR-221 may be counteracted by up-regulated let-7d-3p on their common target genes (ABCA8 and BEND6) after ICH.

Among these miRNA–mRNA pairs, the miR-146a target gene, TLR2, has been extensively studied in inflammation-related diseases including ICH.[11,46] We and others previously reported that TLR2 mRNA along with TLR4 mRNA were increased in blood of ICH patients.[11] Wang et al.[46] demonstrated that TLR2 participated in the hemoglobin-induced inflammation via assembly of TLR2 and TLR4 heterodimers in an experimental ICH model.[46]

Our data also showed that other miR-146a target mRNAs (ROCK1 and ZNF292) were increased in blood of ICH patients. Although ROCK1 or ZNF292 have not previously been reported to be increased in blood of ICH patients, they have been associated with inflammatory processes which produced BBB disruption in subarachnoid hemorrhage (SAH), ischemic stroke and Alzheimer's disease (AD).[47–49] In addition, ROCK1 inhibitors improved outcomes in experimental traumatic brain injury (TBI), stroke, subarachnoid hemorrhage and Alzheimer's Disease models.[48–51]

A recent study showed that miR-210 decreased in blood of ischemic stroke patients[52] as it did here

following ICH. miR-210 was significantly down-regulated in human plasma in patients with unstable carotid stenosis[53] and modulating miR-210 in mice attenuated the infarct size by up-regulating Wnt signaling via APC[53] and suppressing cytokines.[54] miR-210 also modulates neurogenesis and angiogenesis following experimental ischemic stroke,[55,56] and might have similar effects in ICH.

The miR-17-92 cluster, related to miR-17, regulates neurogenesis and angiogenesis during development and following ischemic stroke,[57] but has not been studied following ICH. miRNA from the miR-17-92 cluster in exosomes enhances neuroplasticity and improves outcomes following experimental rodent strokes.[58] miR-17 family members down-regulate mitochondrial anti-oxidant enzymes,[59] modulate NF-κB signaling,[60] and play a role in neuronal apoptosis in hypoxic–ischemic rats.[61] A miR-17 family member is elevated in blood of patients with unstable angina and ST-segment elevated myocardial infarction.[62]

miR-221 increases in CSF following SAH in patients[63] and decreased in whole blood following ICH in this study both at ≤72 h and at >72 h. miR-4514 which decreased at >72 h following ICH, targets the NF-κB inhibitor beta gene which suppresses inflammation.[64] Thus, decreased miR-4514 might promote inflammation following ICH. miR-378 which was also down-regulated following ICH, also regulates inflammatory/ NF-κB pathways.[65,66] miR-378 decreases ischemic neuronal injury by down-regulating caspase 3[67] and modulates neural stem cell proliferation.[68] miR-378 also promotes autophagy and inhibits apoptosis[69] which are important in ICH.

miR-874, decreased at ≤72 h in ICH, can regulate angiogenesis via the STAT-3/ VEGF pathways.[70] miR-874 can also modulate cell death via caspase 8.[71] miR-146 regulates B and T cells[72] and has other effects on the immune system,[73] which likely play a role in ICH. miR-532 regulates hypoxia-induced apoptosis[74] and regulates nitric oxide (NO) release.[75] miR-93 targets ULK1, Nrf2,[76] TLR4[77] and calpain 2[78] and is down-regulated in blood following ICH.

Though not the focus of this study, seven snoRNA (small nucleolar RNA) were shown to change expression in whole blood following ICH. snoRNAs mainly guide methylation and pseudouridylation of ribosomal RNAs, transfer RNAs and small nuclear RNAs, and can sometimes function as miRNAs. One of the regulated snoRNAs in this study (upregulated in ICH > 72 h vs VRFC) HBII-52-13 (aka SNORD115-13) is downregulated in cerebral cavernous malformations[79] (CCM) which are a cause of ICH, and is also involved in alternative splicing,[80] which we have shown is dysregulated in blood following ICH.[11,12]

There are several limitations of the study. First, there were no mRNA that were significantly different between ICH and controls at >72 h. This contrasts with our previous study showing many regulated mRNA at times past 48 h following ICH.[11] However, in this study, the sample size for ICH subjects >72 h was smaller, and stringent statistical criteria were applied which resulted in no mRNA being detected. Relaxation of the FDR statistical threshold results in many mRNA regulated after 72 h as previously reported.[11] Though the miRNA–mRNA pairings are theoretically correct (exact base matches in miRNA and mRNA), there are a variety of factors which may act to prevent the regulation of the mRNA by a miRNA, including alternative polyadenylation sites and alternative splicing which can alter or eliminate binding of a miRNA. Thus, direct proof of miRNA regulation of specific mRNA would be needed in future studies. Moreover, future studies will need to examine which cell type(s) in blood express which miRNA, the gene expression-time response, and the impact of hemorrhage size on expression of various miRNAs/mRNAs. Though we performed cross-validation to evaluate the prediction accuracy of the differentially expressed miRNAs and mRNAs, future studies that use qRT-PCR for identified miRNA and mRNA in an independent validation cohort are needed to evaluate sensitivity and specificity of these diagnostic biomarkers.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' contributions

Xiyuan Cheng performed the studies, analyzed the data, and wrote the manuscript. Bradley P Ander performed the studies, analyzed data and obtained funding. Glen Jickling designed the study, analyzed the data, and reviewed the manuscript. Xinhua Zhan reviewed the manuscript. Heather Hull performed the studies and analyzed data. Frank Sharp designed the study, analyzed the data, wrote the manuscript and obtained funding. Boryana Stamova analyzed the data, wrote the manuscript and obtained funding.

Supplemental material

Supplemental material for this paper can be found at the journal website: http://journals.sagepub.com/home/jcb

References

1. Flaherty ML, Haverbusch M, Sekar P, et al. Long-term mortality after intracerebral hemorrhage. *Neurology* 2006; 66: 1182–1186.
2. Fogelholm R, Murros K, Rissanen A, et al. Long term survival after primary intracerebral haemorrhage: a retrospective population based study. *J Neurol Neurosurg Psychiatry* 2005; 76: 1534–1538.
3. Keep RF, Hua Y and Xi G. Intracerebral haemorrhage: mechanisms of injury and therapeutic targets. *Lancet Neurol* 2012; 11: 720–731.
4. Tang Y, Nee AC, Lu A, et al. Blood genomic expression profile for neuronal injury. *J Cereb Blood Flow Metab* 2003; 23: 310–319.
5. Jakymiw A, Pauley KM, Li S, et al. The role of gw/p-bodies in RNA processing and silencing. *J Cell Sci* 2007; 120: 1317–1323.
6. Wu L, Fan J and Belasco JG. MicroRNAs direct rapid deadenylation of mRNA. *Proc Natl Acad Sci U S A* 2006; 103: 4034–4039.
7. Lim LP, Lau NC, Garrett-Engele P, et al. Microarray analysis shows that some micrornas downregulate large numbers of target mRNAs. *Nature* 2005; 433: 769–773.
8. Liu DZ, Tian Y, Ander BP, et al. Brain and blood microrna expression profiling of ischemic stroke, intracerebral hemorrhage, and kainate seizures. *J Cereb Blood Flow Metab* 2010; 30: 92–101.
9. Sharp FR, Xu H, Lit L, et al. Genomic profiles of stroke in blood. *Stroke* 2007; 38: 691–693.
10. Jickling GC, Liu D, Ander BP, et al. Targeting neutrophils in ischemic stroke: translational insights from experimental studies. *J Cereb Blood Flow Metab* 2015; 35: 888–901.
11. Stamova B, Ander BP, Jickling G, et al. The intracerebral hemorrhage blood transcriptome in humans differs from the ischemic stroke and vascular risk factor control blood transcriptomes. *J Cereb Blood Flow Metab.* Epub ahead of print 13 April 2018. DOI: 10.1177/0271678X18769513.
12. Dykstra-Aiello C, Jickling GC, Ander BP, et al. Intracerebral hemorrhage and ischemic stroke of different etiologies have distinct alternatively spliced mRNA profiles in the blood: a pilot RNA-seq study. *Transl Stroke Res* 2015; 6: 284–289.
13. Griffiths-Jones S, Grocock RJ, van Dongen S, et al. miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic Acids Res* 2006; 34: D140–144.
14. Ezkurdia I, Juan D, Rodriguez JM, et al. Multiple evidence strands suggest that there may be as few as 19,000 human protein-coding genes. *Hum Mol Genet* 2014; 23: 5866–5878.
15. Friedman RC, Farh KK, Burge CB, et al. Most mammalian mrnas are conserved targets of micrornas. *Genome Res* 2009; 19: 92–105.
16. Zhu Y, Wang JL, He ZY, et al. Association of altered serum micrornas with perihematomal edema after acute intracerebral hemorrhage. *PLoS One* 2015; 10: e0133783.
17. Wang MD, Wang Y, Xia YP, et al. High serum mir-130a levels are associated with severe perihematomal edema and predict adverse outcome in acute ich. *Mol Neurobiol* 2016; 53: 1310–1321.
18. Wang J, Zhu Y, Jin F, et al. Differential expression of circulating micrornas in blood and haematoma samples from patients with intracerebral haemorrhage. *J Int Med Res* 2016; 44: 419–432.
19. Hu YL, Wang H, Huang Q, et al. Microrna-23a-3p promotes the perihematomal edema formation after intracerebral hemorrhage via zo-1. *Eur Rev Med Pharmacol Sci* 2018; 22: 2809–2816.
20. Martinez B and Peplow PV. Blood micrornas as potential diagnostic markers for hemorrhagic stroke. *Neural Regen Res* 2017; 12: 13–18.
21. Iwuchukwu I, Nguyen D and Sulaiman W. Microrna profile in cerebrospinal fluid and plasma of patients with spontaneous intracerebral hemorrhage. *CNS Neurosci Ther* 2016; 22: 1015–1018.
22. 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.
23. Zheng HW, Wang YL, Lin JX, et al. Circulating micrornas as potential risk biomarkers for hematoma enlargement after intracerebral hemorrhage. *CNS Neurosci Ther* 2012; 18: 1003–1011.
24. Powers CJ, Dickerson R, Zhang SW, et al. Human cerebrospinal fluid microrna: temporal changes following subarachnoid hemorrhage. *Physiol Genomics* 2016; 48: 361–366.
25. Lu G, Wong MS, Xiong MZQ, et al. Circulating micrornas in delayed cerebral infarction after aneurysmal subarachnoid hemorrhage. *J Am Heart Assoc* 2017; 6: pii: e005363.
26. Arroyo JD, Chevillet JR, Kroh EM, et al. Argonaute2 complexes carry a population of circulating micrornas independent of vesicles in human plasma. *Proc Natl Acad Sci U S A* 2011; 108: 5003–5008.
27. Liu DZ, Ander BP, Tian Y, et al. Integrated analysis of mrna and microrna expression in mature neurons, neural progenitor cells and neuroblastoma cells. *Gene* 2012; 495: 120–127.
28. Paquette J and Tokuyasu T. Egan: exploratory gene association networks. *Bioinformatics* 2010; 26: 285–286.
29. Agarwal V, Bell GW, Nam JW, et al. Predicting effective microrna target sites in mammalian mRNAs. *Elife.* Epub ahead of print 12 April 2015. DOI: 10.7554/eLife.05005.
30. Hsu SD, Tseng YT, Shrestha S, et al. Mirtarbase update 2014: an information resource for experimentally validated mirna-target interactions. *Nucleic Acids Res* 2014; 42: D78–85.
31. Chou CH, Shrestha S, Yang CD, et al. Mirtarbase update 2018: a resource for experimentally validated microrna-target interactions. *Nucleic Acids Res* 2018; 46: D296–D302.

32. Stamova B, Xu H, Jickling G, et al. Gene expression profiling of blood for the prediction of ischemic stroke. *Stroke* 2010; 41: 2171–2177.

33. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009; 136: 215–233.

34. Betel D, Koppal A, Agius P, et al. Comprehensive modeling of microrna targets predicts functional non-conserved and non-canonical sites. *Genome Biol* 2010; 11: R90.

35. Liu DZ, Stamova B, Hu S, et al. MicroRNA and mRNA expression changes in steroid naive and steroid treated DMD patients. *J Neuromuscul Dis* 2015; 2: 387–396.

36. Turchinovich A, Samatov TR, Tonevitsky AG, et al. Circulating miRNAs: cell-cell communication function? *Front Genet* 2013; 4: 119.

37. Turchinovich A, Weiz L, Langheinz A, et al. Characterization of extracellular circulating microrna. *Nucleic Acids Res* 2011; 39: 7223–7233.

38. Williams Z, Ben-Dov IZ, Elias R, et al. Comprehensive profiling of circulating microRNA via small RNA sequencing of cDNA libraries reveals biomarker potential and limitations. *Proc Natl Acad Sci U S A* 2013; 110: 4255–4260.

39. Jickling GC, Ander BP, Shroff N, et al. Leukocyte response is regulated by microrna let7i in patients with acute ischemic stroke. *Neurology* 2016; 87: 2198–2205.

40. Zhang J, Xu X, Zhao S, et al. The expression and significance of the plasma let-7 family in anti-n-methyl-d-aspartate receptor encephalitis. *J Mol Neurosci* 2015; 56: 531–539.

41. Li Y and Zhang Z. Computational biology in microRNA. *Wiley Interdiscip Rev RNA* 2015; 6: 435–452.

42. Lu J and Clark AG. Impact of microRNA regulation on variation in human gene expression. *Genome Res* 2012; 22: 1243–1254.

43. Jansen BJ, Sama IE, Eleveld-Trancikova D, et al. Microrna genes preferentially expressed in dendritic cells contain sites for conserved transcription factor binding motifs in their promoters. *BMC Genom* 2011; 12: 330.

44. Xu B, Huang Y, Niu X, et al. Hsa-mir-146a-5p modulates androgen-independent prostate cancer cells apoptosis by targeting rock1. *Prostate* 2015; 75: 1896–1903.

45. Zhang B, Wang A, Xia C, et al. A single nucleotide polymorphism in primary-microrna-146a reduces the expression of mature microrna-146a in patients with Alzheimer's disease and is associated with the pathogenesis of Alzheimer's disease. *Mol Med Rep* 2015; 12: 4037–4042.

46. Wang YC, Zhou Y, Fang H, et al. Toll-like receptor 2/4 heterodimer mediates inflammatory injury in intracerebral hemorrhage. *Ann Neurol* 2014; 75: 876–889.

47. Shi Y, Zhang L, Pu H, et al. Rapid endothelial cytoskeletal reorganization enables early blood-brain barrier disruption and long-term ischaemic reperfusion brain injury. *Nat Commun* 2016; 7: 10523.

48. Lee LL, Aung HH, Wilson DW, et al. Triglyceride-rich lipoprotein lipolysis products increase blood-brain barrier transfer coefficient and induce astrocyte lipid droplets and cell stress. *Am J Physiol Cell Physiol* 2017; 312: C500–C516.

49. Naraoka M, Munakata A, Matsuda N, et al. Suppression of the rho/rho-kinase pathway and prevention of cerebral vasospasm by combination treatment with statin and fasudil after subarachnoid hemorrhage in rabbit. *Transl Stroke Res* 2013; 4: 368–374.

50. Fujii M, Duris K, Altay O, et al. Inhibition of rho kinase by hydroxyfasudil attenuates brain edema after subarachnoid hemorrhage in rats. *Neurochem Int* 2012; 60: 327–333.

51. Liu DZ, Sharp FR, Van KC, et al. Inhibition of src family kinases protects hippocampal neurons and improves cognitive function after traumatic brain injury. *J Neurotrauma* 2014; 31: 1268–1276.

52. Zeng L, Liu J, Wang Y, et al. Microrna-210 as a novel blood biomarker in acute cerebral ischemia. *Front Biosci* 2011; 3: 1265–1272.

53. Eken SM, Jin H, Chernogubova E, et al. Microrna-210 enhances fibrous cap stability in advanced atherosclerotic lesions. *Circ Res* 2017; 120: 633–644.

54. Huang L, Ma Q, Li Y, et al. Inhibition of microrna-210 suppresses pro-inflammatory response and reduces acute brain injury of ischemic stroke in mice. *Exp Neurol* 2018; 300: 41–50.

55. Voloboueva LA, Sun X, Xu L, et al. Distinct effects of mir-210 reduction on neurogenesis: increased neuronal survival of inflammation but reduced proliferation associated with mitochondrial enhancement. *J Neurosci* 2017; 37: 3072–3084.

56. Lou YL, Guo F, Liu F, et al. Mir-210 activates notch signaling pathway in angiogenesis induced by cerebral ischemia. *Mol Cell Biochem* 2012; 370: 45–51.

57. Yang P, Cai L, Zhang G, et al. The role of the mir-17-92 cluster in neurogenesis and angiogenesis in the central nervous system of adults. *J Neurosci Res* 2017; 95: 1574–1581.

58. Xin H, Katakowski M, Wang F, et al. Microrna cluster mir-17-92 cluster in exosomes enhance neuroplasticity and functional recovery after stroke in rats. *Stroke* 2017; 48: 747–753.

59. Xu Z, Zhang Y, Ding J, et al. Mir-17-3p downregulates mitochondrial antioxidant enzymes and enhances the radiosensitivity of prostate cancer cells. *Mol Ther Nucleic Acids* 2018; 13: 64–77.

60. Liu F, Cheng L, Xu J, et al. Mir-17-92 functions as an oncogene and modulates nf-kappab signaling by targeting traf3 in mgc-803 human gastric cancer cells. *Int J Oncol* 2018; 53: 2241–2257.

61. Gamdzyk M, Doycheva DM, Malaguit J, et al. Role of PPAR-beta/delta/miR-17/TXNIP pathway in neuronal apoptosis after neonatal hypoxic-ischemic injury in rats. *Neuropharmacology* 2018; 140: 150–161.

62. Zhong Z, Hou J, Zhang Q, et al. Circulating microrna expression profiling and bioinformatics analysis of dysregulated microRNAs of patients with coronary artery disease. *Medicine* 2018; 97: e11428.

63. Bache S, Rasmussen R, Rossing M, et al. Microrna changes in cerebrospinal fluid after subarachnoid hemorrhage. *Stroke* 2017; 48: 2391–2398.

64. Othumpangat S, Bryan NB, Beezhold DH, et al. Upregulation of mirna-4776 in influenza virus infected

bronchial epithelial cells is associated with downregulation of NFKBIB and increased viral survival. *Viruses* 2017; 9: E94. DOI: 10.3390/v9050094.

65. Zhang T, Hu J, Wang X, et al. Microrna-378 promotes hepatic inflammation and fibrosis via modulation of the NF-kB-TNFalpha pathway. *J Hepatol* 2019; 70: 87–96.

66. Caserta S, Mengozzi M, Kern F, et al. Severity of systemic inflammatory response syndrome affects the blood levels of circulating inflammatory-relevant micrornas. *Front Immunol* 2017; 8: 1977.

67. Zhang N, Zhong J, Han S, et al. MicroRNA-378 alleviates cerebral ischemic injury by negatively regulating apoptosis executioner caspase-3. *Int J Mol Sci* 2016; 17(pii): E1427.

68. Huang Y, Liu X and Wang Y. Microrna-378 regulates neural stem cell proliferation and differentiation in vitro by modulating tailless expression. *Biochem Biophys Res Commun* 2015; 466: 214–220.

69. Li Y, Jiang J, Liu W, et al. MicroRNA-378 promotes autophagy and inhibits apoptosis in skeletal muscle. *Proc Natl Acad Sci U S A* 2018; 115: E10849–E10858.

70. Zhang X, Tang J, Zhi X, et al. miR-874 functions as a tumor suppressor by inhibiting angiogenesis through STAT3/VEGF-A pathway in gastric cancer. *Oncotarget* 2015; 6: 1605–1617.

71. Wang K, Liu F, Zhou LY, et al. Mir-874 regulates myocardial necrosis by targeting caspase-8. *Cell Death Dis* 2013; 4: e709.

72. Cho S, Lee HM, Yu IS, et al. Differential cell-intrinsic regulations of germinal center b and t cells by miR-146a and mir-146b. *Nat Commun* 2018; 9: 2757.

73. Testa U, Pelosi E, Castelli G, et al. Mir-146 and mir-155: two key modulators of immune response and tumor development. *Noncoding RNA* 2017; 3: E22. DOI: 10.3390/ncrna3030022.

74. Ma J, Zhang J, Wang Y, et al. Mir-532-5p alleviates hypoxia-induced cardiomyocyte apoptosis by targeting pdcd4. *Gene* 2018; 675: 36–43.

75. Qin JZ, Wang SJ and Xia C. Micrornas regulate nitric oxide release from endothelial cells by targeting nos3. *J Thromb Thrombol* 2018; 46: 275–282.

76. Yan LJ, Fan XW, Yang HT, et al. Mir-93 inhibition ameliorates ogd/r induced cardiomyocyte apoptosis by targeting nrf2. *Eur Rev Med Pharmacol Sci* 2017; 21: 5456–5461.

77. Li Y, Wang J, Sun L, et al. Lncrna myocardial infarction-associated transcript (miat) contributed to cardiac hypertrophy by regulating tlr4 via mir-93. *Eur J Pharmacol* 2018; 818: 508–517.

78. Yang SJ, Wang J, Xu J, et al. Mir-93mediated collagen expression in stress urinary incontinence via calpain-2. *Mol Med Rep* 2018; 17: 624–629.

79. Kar S, Bali KK, Baisantry A, et al. Genome-wide sequencing reveals small nucleolar RNAs downregulated in cerebral cavernous malformations. *Cell Mol Neurobiol* 2018; 38(7): 1369–1382.

80. Kishore S, Khanna A, Zhang Z, et al. The snoRNA MBII-52 (snord 115) is processed into smaller RNAs and regulates alternative splicing. *Hum Mol Genet* 2010; 19: 1153–1164.